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RESEARCH BULLETIN NO. 1

UNIVERSITY OF MISSOURI

COLLEGE OF AGRICULTURE

AGRICULTURAL EXPERIMENT STATION

An Experimental Study of the Rest Period in Plants

The Winter Rest

FIRST REPORT

COLUMBIA, MISSOURI
April, 1910

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[1] In the service of the U. S. Department of Agriculture.

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AN EXPERIMENTAL STUDY OF THE REST PERIOD IN PLANTS.

THE WINTER REST: INITIAL REPORT ON THE TREATMENT OF DORMANT WOODY PLANTS FOR FORCING THEM INTO GROWTH.

By W. L. HOWARD, *Professor of Horticulture.*

BRIEF SUMMARY

(1) Plants rest in winter, and they also have rest periods in summer, but all of these forms of rest are caused by unfavorable external conditions.

(2) Outward conditions determine both the time of occurrence and the degree of intensity of the period of rest. If unfavorable conditions, such as cold or drought, occur at regular intervals, a plant readily adapts itself to the new demands and the rest becomes a habit which may continue to be repeated automatically for a longer or shorter period of time.

(3) The habit of rest induced by outward conditions is often very strongly fixed and is apparently transmissible.

(4) There are many ways of arousing plants from a resting state (especially the winter rest), the principal ones known being the raising of the temperature, the use of gases and vapors, by freezing, and through desiccation.

(5) By means of careful cultural methods plants may be caused to omit their ordinary habit of resting and grow continuously.

(6) There are numerous theories of the workings of some of the special treatments, such as ether and chloroform, freezing and drying, and how they produce growth, but it is probable that none of them exert any specific action.

Introduction

The work done by the Missouri Experiment Station[1] on the hardiness of the peach, covering the past several years, shows in emphatic manner that, fundamentally, the matter of hardiness (with this fruit at least) is intimately associated with the rest period. In order, therefore, to arrive at a better understanding of this aspect of

[1] Whitten, J. C., Winter protection of the peach. Bul. No. 38, Mo. Exp. Sta. 1897; and Chandler, W. H., The hardiness of the peach: Winter killing of the buds as influenced by previous treatment. Bul. No. 74, Mo. Exp. Sta. 1907.

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the subject of hardiness, it was deemed necessary to make a careful study of the nature and principles of the rest period of plants in general.

By "rest period" or "dormant period" is meant the time of rest, or period when plants do not grow. This, as a rule, corresponds to the winter season of the year with most of our common woody plants that grow outside in this climate. It is a matter of common experience that many of the plants that rest or remain inactive during the winter, will begin to grow in a short time if taken into a warm room, even in midwinter. Some will even begin to grow outside during the winter after the weather has been mild for a few days. On the other hand, there are many plants that will make no growth whatever until near spring, even though they are taken into a warm, moist room and kept there. These experiences led to the popular belief that there are many plants which have such profound resting periods that they cannot be made to grow until at or near the normal time for awakening in spring.

In order to learn something of the fundamental nature of the rest period and to test the extent to which plants may be caused to start into growth out of their season, i. e. while dormant, nearly three hundred species of woody plants were examined by bringing them into the greenhouse after they had gone into their resting state, and noting which ones began to grow under the influence of the warmth. As anticipated, it was found that the dormant state varied very greatly with the different species, not only in point of the duration of the rest, but also in the degree of its intensity. The extremes in woody plants are probably represented by two typical cases, viz., *Spiraea sorbifolia* L. and *Fagus sylvatica* L. The former is a plant which appears to have no resting period, seeming to stop growth only when the weather is actually too cold for cell division, while the latter goes dormant in the fall at the approach of cold weather and remains so until the time of settled weather in spring, even though it be brought into a warm, moist room. In short, the *Spiraea* will make an almost continuous growth during the winter when kept inside or, will spring into growth within a day if brought in during the winter, while the *Fagus* will not grow at all during its period of rest through the influence of warmth alone.

Out of the long list experimented with, quite a number were found to be very difficult to awaken from their resting state, even under severe treatment, while others would eventually make some growth merely under the influence of warmth, but would grow much more readily when given special treatment. Experiments were then

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inaugurated to test the efficacy of certain treatments for forcing growth.

As a result of extensive studies and experimentation, it is concluded that nearly all of our ordinary woody plants, such as fruit trees, ornamental shrubs, etc., have definite resting periods, that is, that each species, and possibly each variety, has a certain number of days during which it will not grow under natural conditions. These facts have led to the conclusion that a knowledge of the so-called winter rest period of plants, its nature and extent in different kinds and types of plants, is fundamental to the study of many of the practical questions of horticulture.

One practical application of the above principle is that, if, say, in the case of the peach, which is known to have a comparatively short rest period (about six weeks), the trees can be caused to continue growing later in the autumn, that is, go dormant late, they will remain dormant a correspondingly longer time in late winter, and hence not be so liable to injury by freezing after having made a slight growth. Peaches are nearly always killed by reason of their habit of premature growth during warm days in late winter. Every fruit grower knows that there is no danger of growth taking place in early winter, no matter how warm and mild the weather may be, because the trees are then in the midst of their rest period. When fully dormant, peach buds can safely withstand the low temperature of 20 degrees F., but after having made a slight growth, zero weather will nearly always be sufficient to kill them all.

The subject of hardiness, then, in the peach, as well as in other plants, may be found to be intimately associated with the rest period. If it were known definitely that plants have a tendency to grow at unseasonable times by reason of the fact that they have very slight resting periods, or that the resting stage is at an end, we might then set about devising means for regulating the period of dormancy, or, knowing that their habits of growth in this respect cannot be easily changed, of finding means for protecting them.

Another practical advantage resulting from a knowledge of the rest period of plants, together with a practical means for rousing them into growth out of season, is in the commercial forcing of flowering plants and garden vegetables, as practiced by the florist and gardener. Large sums of money are now invested in plants like lilac, deutzia, etc., they being grown especially for forcing purposes in winter. The use of anesthetics (ether and chloroform), as well as freezing and desiccation, in causing these plants to grow quickly and bloom profusely, will undoubtedly soon play a very important part, if indeed they do not supplant other methods of forcing. Vegetables, like rhu-

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barb and asparagus, have been forced by the use of anesthetics in a commercial way, to only a comparatively slight extent, although with the improvement in methods of handling, anesthetics and also desiccation and freezing may be used to a much larger extent in this sort of forcing work.

GENERAL REVIEW OF EXPERIMENTS AND RESULTS.

Owing to the very slight amount of experimental data available concerning the dormancy of plants, it was found necessary, at the outset, to test as many species—both trees and shrubs—as possible, to find if they could be made to grow during winter. All the plants used in the main experiments were deciduous species, native to the temperate zone. All of them passed into their usual state of dormancy at the approach of cold weather. This was about the middle of October.

Taking the plants, then, as they occurred under their environment, the first question to be answered was, which ones could be aroused into growth during the early stage of their dormancy, by any means whatsoever. Many, it was known, would grow with comparative ease, merely under the influence of warmth, while there were others that would make no growth whatever under such circumstances. Also some data were available showing that certain treatments—particularly with ether and chloroform, were effective in hastening growth in the cases of the few plants that have been tried, but to what extent these or any other treatments would hasten growth or, produce growth at all, with the great majority of plants, was practically unknown.

The investigation was begun in the fall of 1905 at the Botanical Institute of the University, at Halle, Germany. The Botanic Garden there contained a good collection of trees and shrubs growing under natural conditions for the work. Not only was the collection of the German and other European species large, but it included many forms from the United States and other countries, that were especially useful for the experiment. These plants being of wide distribution, offered special opportunity for comparing results obtained with the results of similar tests carried on at the Missouri Experiment Station the following year—1906-7.

From the outset positive results were quickly forthcoming and, for the most part, the main question of the investigation was answered. The first list of 283 species was not treated, the plants being merely brought into the warm, moist greenhouse, where they were allowed to stay until they either grew or died. The list mentioned comprised two collections, one made in November and the other in

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January. Of the first collection over half grew and from the last 86 per cent grew.

After this preliminary investigation special lists were made up to be treated for forcing growth. As a rule, those species that had been found to grow easily without treatment were omitted. The principal treatments employed were ether, freezing, drying, and confining in a moist dark-chamber. These treatments were used both singly and in combination. There were three main experiments and several more or less isolated miscellaneous tests.

In all, during the three main experiments, between November 17 and December 24, 1905 (Tables III, VI and IX), 133 different species were treated and all but 14 grew. By various means employed in some of the miscellaneous tests, 7 out of the 14 were caused to grow, so that growth was secured in January or earlier, from 126 species. Of the remaining 7, *Liriodendron tulipifera* and *Fagus sylvatica* grew in February, and *Carya aquatica, C. porcina, Juglans regia, Quercus alba* and *Q. coccinea,* in March.

It should be noted also that of the 283 species that were merely brought into the greenhouse (not treated), 211 grew in January or earlier, 36 by February or before, and the remaining 36 by March or earlier.

The real importance of this information lies not so much in the matter of whether the plants grew with or without treatment, as in the fact that they would grow at all by mid-winter or before. *This shows conclusively, that the great majority of species, indigenous to temperate climates, do not have a firmly fixed winter resting period from which they cannot be awakened.*

It is of interest that more than half of the entire list of kinds grew easily inside of two weeks, without treatment. The remaining 140 or 150 forms, however, awakened with more or less difficulty. Seventy-two of the kinds showed much resistance to growth even in late winter (February) and 36 of the number were very resistant and would not grow (untreated) until the approach of spring (March).

The species found most difficult to force were *Carya aquatica, C. porcina, Fagus sylvatica, Fraxinus americana, F. excelsior, F. Ornus, Juglans regia, Liriodendron tulipifera, Quercus alba, Q. coccinea* and *Q. olivaeformis.* Seven of this list with strong resting periods are American species and four of European and Asiatic origin.

Experience in this investigation has shown that there are apparently times when a given species will grow more readily than at other times under treatment, which leads to the belief that if the treatments are repeated at frequent intervals it is highly probable that all of the

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species may be forced into growth in early or mid-winter. From the experiments of Klebs [2] we are justified in the opinion that with previous cultural treatment the most resistant forms can be made to grow at any time and perhaps eventually be made to have very short, or light, resting periods, if not to omit them entirely.

DISCUSSION OF THE REST PERIOD.

Since our common plants in nature have a regularly recurring dormant stage, the question arises as to what is the nature of this rest period. Pfeffer [3] has cited Askenasy's experiments with a large number of plants including *Quercus pedunculata, Tilia europea,* and *Castanea vesca,* stating that they and other species would not open their buds before March or April and that some of them required even a longer period of rest. Klebs,[4] however, dissented from this opinion, but the citations given above show the prevalence of the belief that in a great many plants the rest period of winter is long and deep and that it cannot be broken. The experiments of the writer, too, show that such a view is incorrect. The species mentioned by Pfeffer and Askenasy were said not to grow before March or April, but the results of the experiments herein reported show that they will grow from treatments in early December and without treatment in January.

But why do they become dormant? Is it necessary that they cease all growth for a time, or do they do so because they are overtaken by winter? In other words, do the plants voluntarily become dormant or are they forced into that state on account of unfavorable conditions?

The first case would be the result of an inward, strongly fixed, hereditary law, while the other would only occur—if the inherent tendencies of the plants could be followed—when outside conditions brought it about. That the ordinary winter rest is firmly fixed is experimentally disproven but it will be appropriate here to discuss the subject further. Pfeffer believed that there were two forms of rest periods of plants, the one due to inward causes, which he called "autonomic" and the other the result of outward conditions, which (in 1901) he termed "aitionomic." Concerning this view he later discussed [5] the rise and fall of growth in certain plants and his con-

[2] Klebs, Georg, Willkürliche Entwickelungsänderungen bei Pflanzen. pp. 127-137.

[3] Pfeffer, W., Pflanzenphysiologie. Bd. II. 1904. p. 260.

[4] Klebs, Georg, Willkürliche Entwickelungsänderungen bei Pflanzen, p. 129.

[5] Pfeffer, W., Pflanzenphysiologie. Bd. II. 1904. p. 20.

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clusions were that, inasmuch as the oscillations occurred during the most uniform outward conditions, they were due to autonomic causes. As the result of years of direct experimenting with *Sempervivum* and other plants, Klebs[6] did not agree with Pfeffer, and in 1905, in discussing the question he declared that, while there was a difference in the kind and degree of variability in plants, and that to a greater or less extent this, as well as all other variations or changes in plants were brought about by the inner conditions, these inner conditions were wholly dependent upon the environment and hence a change in outer conditions, or rather the character of the outer conditions, would determine the changes that take place in the plant.

From his own investigations, and now by the writer's the position taken by Klebs seems very probable,—that all rest periods of vegetation are traceable to one cause, and, that that cause is environment, seems clear.

Returning to the matter of the dormant stage of woody plants, it should be stated that the approach of the rest period is usually preceded by the formation of terminal buds on all growing shoots and (with deciduous plants) the shedding of the leaves. This preparation for winter occurs with great regularity every year in any given place, but there is much good evidence to show that the plants would not become dormant if not compelled to do so by the cold.

In the first place, the large number of species in the writer's experiments in forcing—more than 50 per cent of the number tested—which grew readily when brought into the warm air, shows that the rest was abnormal, and the experiments of Klebs[7] with annual, biennial and perennial herbaceous plants show in conclusive manner the astonishing fact that they not only would not become dormant if not forced to do so by the winter, but they did not require rest.

Although unnecessary, the oft-repeated habit of becoming dormant at a particular time, year after year, exerts a powerful influence upon a plant and even causes the habit to become so firmly fixed as to be transmissible. An example of this is shown in some northern and southern grown forest tree seeds of different species, particularly *Gleditschia triacanthos, Acer negundo,* and *Juglans nigra,* which were sown on the Horticultural Grounds of the Missouri Experiment Station in 1896. At first there were notable differences in the time of leaf-fall and rate of growth between those from the different regions, but after eight or nine years these differences, together with others,

[6] Klebs, Georg, Ueber Variationen der Blüten. p. 297.
[7] Klebs, Georg, Willkürliche Entwickelungsänderungen bei Pflanzen. pp. 129-138.

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had adjusted themselves to correspond with their new environment. This example furnishes experimental proof that environment may be much stronger than heredity, i. e. that characters which are so firmly fixed as to be transmitted to the seed can be overcome and changed in a comparatively short time by external conditions. In this connection DeCandolle,[8] writing a hundred years ago, stated that plants from southern climates strive to repeat their normal periodicity, and if the external conditions permit it, develop leaves and flowers at unusual times, but in the progress of a few years, however, they may accommodate themselves to the new conditions and by lengthening their resting period, assume a yearly periodicity corresponding to the changed climate.

Plants are plastic enough to quickly adjust themselves to new conditions, especially when their lives are endangered, while otherwise the change may take place slowly. An illustration of the first case was the southern plants that were taken north and at first winter killed, while a good example of the latter case is the following: Two or three specimens of *Quercus pedunculata,* growing in pots were kept in the greenhouse both summer and winter for two years and they still continued to cease growing in autumn and remain dormant until spring, thus showing that the habit of resting for a long period during winter was strongly fixed. But this strong rest period can be broken, even in its early stage, as is shown in the experiment described on page 80, where apparently, slight freezing was the cause of the growth.

The history of the pot-grown *Quercus pedunculata,* mentioned above, is here of interest. In the autumn of 1904 Professor Klebs planted the acorns in pots, which were kept in the warm greenhouse. The seed soon germinated and the young plants grew vigorously all winter and until the following March, when winter buds were formed and all growth ceased until early summer. This experience is identical with the behavior of the oaks that were forced into growth in winter by freezing and etherizing. (See pp. 23 and 80.)

That the winter rest may be broken readily in a great many species where the habit is strongly fixed has been shown in the experimental data of this report, so that it will now be instructive to notice the experiments of Flammarion[9] and Klebs. In 1891 the former planted a number of acorns of *Quercus robur* in pots and kept them protected from winter conditions for fifteen years, with the

[8] DeCandolle, A., Mémoires présentés par divers savants. t. I. 1806. p. 349.

[9] Flammarion, C., Bul. Mens. de l'Off. de Renseigm. Agric. 6, No. 11, pp. 1327-8.

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result that the young trees have changed their habit of growth until they resemble evergreen species, in that the young leaves now appear before the fall of the old ones.

Klebs [10] reported some remarkable results in handling plants to prevent the rest period from taking place. Two typical examples of this work were with *Hyacinthus* and *Ficaria ranunculoides*. The former was caused to grow throughout the summer and flower in September, after having bloomed normally the previous winter. It is the habit of the *Ficaria* to bloom in spring and then the tubers rest throughout the summer and winter until spring again, but by cultivating them in the hotbed and greenhouse they were caused to bloom in winter; then when the plants died down the tubers were again cultivated and by July were once more in bloom.

Regarding these very important results Klebs [11] writes: "In the cases of both the *Hyacinthus* and *Ficaria* it is evident that through the high temperature the inner conditions of the otherwise resting organs were changed so that, on account of the continued favorable conditions, they did not enter the resting period at all, or only partially, and that by further perfecting methods of culture, it will later be possible to bring the majority of plants with inner rest period to an uninterrupted growth."

The foregoing results throw much light upon the subject of the rest period. The experiment last mentioned, with the conclusions reached by the investigator are most important for, if by cultural methods, or other previous preparation, the usual rest period is omitted entirely, or at least much attenuated, then the most refractory species (*Hicoria, Quercus, Fagus,* etc.) may be made to grow throughout the winter (in a greenhouse), or, certainly to be awakened at will. Further investigation on this point will be awaited with interest.

The foregoing statements and examples indicate that the winter rest is a habit induced by unfavorable outward circumstances and that, if those conditions are removed, some species at least while yet under the powerfully acting influence of recent habit, can be induced to grow readily. That outward conditions may profoundly modify the habits of plants was shown in the case of the northern and southern trees which took on characters intermediate between the two extremes.

[10] Klebs, Georg, Willkürliche Entwickelungsänderungen bei Pflanzen. pp. 129-134.

[11] Klebs, Georg, Willkürliche Entwickelungsänderungen bei Pflanzen. p. 137.

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In his instructive discussion of the relation of the specific structure of plant organisms to their inner and outer conditions, Klebs,[12] after enumerating the factors which constitute the inner conditions, says, "Everything about a cell, an organ or an entire plant is really determined by the inner condition or specific structure of the cell." Continuing, however, he states further that "the fundamental fact upon which the whole biological inquiry rests is the dependence of the inner conditions upon the environment. All of the above mentioned conditions (water content, osmotic pressure, etc.) are variable; but their variability has necessarily a close connection with the environment." Pursuing the subject further, the same author sums up the matter in a single sentence and in so doing really sounds the keynote to the whole subject of variation as brought about by environment. *"It is only necessary,"* said he, *"to influence the cell at the precise time when it is about to assume its definite specific structure or characteristics."*

It is true that the immediate preparation for the winter rest—formation of terminal buds and leaf-fall—comes from within, but all of this is the result of habits formed from oft-repeated occurrences, which are the influencing outward conditions, such as the coming of winter.

The rest period is referred to by Drude and Naumann,[13] Johannsen,[14] and others, as being due to inner causes, but it would evidently be more correct to say that it is caused by outward conditions. The case of the oaks becoming dormant and remaining so in the warm greenhouse may seem to refute this, but when it is remembered that others yielded to treatment and made a luxuriant growth in an early stage of their dormancy, the true nature of the rest period appears to be established.

Besides the winter rest, such as has just been described, there is yet another kind of rest which occurs in summer. There are two forms of summer rest; that caused by drought or injury, and that that is manifested by *Crocus, Anemone, Ficaria,* etc. An example of the first case is furnished by a young elm tree (*Ulmus americana*) which was planted on the Horticultural Grounds of the Missouri Experiment Station in the fall of 1903. The following season the tree made a good growth up to the beginning of August, at which time a drought set in and continued with such severity that it ceased to

[12] Klebs, Georg, Ueber Variationen der Blüten. 1905. pp. 293-4.

[13] Drude, O., Naumann, A., u. Ledien, F., Ueber die von Ostern 1901 bis 1902 im Königl. Botanischen Garten zu Dresden angestellten, den Gartenbau betreffenden Versuche, etc. p. 2.

[14] Johannsen, W., Das Aetherverfahren beim Frühtreiben mit besonderer Berücksichtigung der Fliedertreiberei. 1906. p. 10.

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grow and by September first had formed strong terminal buds, as well as plump, well formed axillary buds, and finally shed its leaves. Heavy rains coming on later, the rest was broken and the buds began to grow. The terminal buds grew and the shoots elongated several inches and in some instances the side buds also grew. Growth continued throughout October and until far into November, the autumn being late, when the leaves fell and the entire tree became dormant in a normal manner, another crop of plump buds being formed in the axils of all the new leaves. It was interesting to note that the twigs showed the usual rings at the points where the second growth began, these being identical with the usual annual markings of this character. A similar experience is reported by P. Magnus [15] of the abnormal growth of an *Aesculus Hippocastanum* in Berlin during the autumn of 1884 where, through heat, drought and injury by insects, the tree closed its growth, shed its leaves and matured its buds, but grew again later when rainy weather came. He also observed the same phenomena in linden trees.

This is clearly an enforced form of rest, having been forced upon the plants by the unfavorable conditions. It is probable, though, that if they were repeated regularly every year, the habit would eventually become strongly fixed with many species.

Schimper [16] and Volkens [17] corrected the popular idea that all tropical vegetation is evergreen. These authors report that at certain times of the year many tropical trees shed their leaves and pass into a state of rest.

Plants apparently have several rest periods. In temperate regions it is difficult to observe this character owing to the disturbing factor of winter. In such regions the well marked seasons determine the time of rest and activity of nearly all forms grown in the open. In the tropics, however, especially in regions of abundant and well distributed rainfall, we might expect to find a true state of affairs as regards the manifestation of a well marked periodicity in plants. Concerning this phenomenon Schimper [18] reports that his observations in tropical districts, with abundance of rain at all seasons of the year, have taught him that there, also, vital processes in plants exhibit a rythmic alternation of periods of repose and activity. This opinion, he declared, however, was dependent upon a conception of rest pe-

[15] Magnus, P., Botanische Mitteilungen. Separatabzug aus den Verhandlungen des Botanischen Vereins der Provinz Brandenburg. XXVI. 1885.

[16] Schimper, A. F. W., Pflanzengeographie auf physiologischer Grundlage. pp. 286 and 370.

[17] Volkens, G., Der Laubwechsel tropischer Bäume. Gartenflora, 52. Jahrg. Heft 22. 1903. pp. 591-598.

[18] Schimper, A. F. W., Plant Geography. Tr. by Fisher, Groom & Balfour. 1903. pp. 241-5.

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riods that would differ from the usual one. By this he meant that there are no periods of rest for the vital processes as a whole, but only resting periods for certain functions. He continues: "A plant during its hibernation is by no means inactive. In many trees, starch is converted into oil; the chlorophyll-corpuscles of conifers produce red coloring matter; the epidermis of the leaves of many herbs produces cyanophyll; the roots continue to grow in length; the winter buds, by means of invisible processes, acquire the power of further development, which was lacking in them in the warm season, and so on. On the other hand, repose chiefly prevails as regards the inception, the growth in length and thickness of the shoots, although there are exceptions. Assimilation is weakened, yet suspended only in hard, frosty weather."

The same author states that: "There is therefore in the temperate zones no season of the year that does not tend to set in motion certain functions of plant-life, and to set others at rest. During autumn and winter repose predominates, and during spring and summer activity predominates, so that we may speak of relative seasons of rest and of vegetation, although at no season is either condition actually realized. Tropical plants are just as subject to the periodic alternation of rest and of activity as are those of the cooler and cold zones. Wherever a sharp climatic periodicity prevails the functions of the plant-organism in the tropics also appears to be decidedly influenced by it. Thus dry seasons act like cold ones in many respects. The less marked the periodicity of the climate is, the less dependent upon its influence is the periodicity in the plant. Internal causes are mainly or solely responsible for the alternation of rest and of activity in a nearly uniform climate. Such a rythmic change is, however, never abandoned, for it arises from the nature of the living organism and not from external conditions; its connection with external conditions is a secondary feature—an adaptation."

As to the detailed behavior of the vegetation where heat, cold or dryness do not interfere, he writes these significant words: "In all tropical districts with very weak climatic periodicity, there are *woody plants that shed their leaves at longer or shorter intervals* (*one to six times a year*), *without any connection with the season of the year*, so that trees of one and the same species, under the same external conditions, acquire fresh foliage and shed their leaves at times that do not agree."

Volkens found that while several species shed their leaves, the period of rest was very short, in some instances new leaves beginning to form as soon as the old ones were off. He observed also that some trees have two periods of leaf-fall during the year. He further states that the time of leaf-fall varies with the different species.

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While general observations on the behavior of tropical vegetation have disclosed some interesting results, it is evident that it is very necessary that exact experiments should be performed before we can draw any definite conclusions as to the causes of leaf-fall at different times of the year. It must be that there are good reasons for the shedding of the leaves at irregular intervals, which, at present, are unknown. Inasmuch as there is no regularity about the occurrence of the defoliation, it stands to reason that there must be influencing factors that have not yet been noticed. Under conditions of great uniformity as regards warmth and moisture, it would not be at all surprising—indeed might be expected—that very slight causes might influence the plants sufficiently to bring about considerable fluctuations in leaf-fall. However, in the absence of more exact physiological data on tropical vegetation, no final explanation can be given for the phenomena mentioned.

Regarding the other form of summer rest, that of the *Anemone, Crocus, Ficaria,* etc., it may be said in general that such plants bloom in spring, then die down, the fleshy underground parts remaining dormant for from seven to ten months, or until spring comes again. Since this phenomenon occurs annually, under the conditions that prevail each year, it might appear at first sight that it was "autonomic" in character, but the experiments of Klebs before referred to with *Ficaria, Glechoma, Ajuga,* etc., indicate that such plants may not only be forced into growth by careful handling during their resting stage, but they may be *kept growing continuously all the year.* This proof then, results in the conclusion that for this class of plants the rest is in some way due to outward conditions (their requirements being different from those of woody plants), and thus similar to the winter rest and to the other forms of enforced summer rest.

We can now look at plants as organisms disposed to have an almost unbroken growth and only hindered from doing so by external conditions. The so-called "Malta" potatoes are said to have no resting period, that is, will grow at any time during the year when conditions are favorable. Schmid [19] planted, on October 21, as early and as late varieties of potatoes as he could procure. Both kinds grew and formed new tubers, and, on November 8, the new crops were harvested and immediately planted. These in turn grew and the new ones were again planted April 6 and these also grew. He had the same results with onions in autumn and winter, except he reports the latter grew even more readily than the potatoes.

[19] Schmid, B., Ueber die Ruheperiode der Kartoffelknollen. Bericht. d. Deutch. Bot. Gesellsch. Bd. XIX. Heft 2.

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Klebs[20] also reports the same results with the sort of potato known as "Maercker." In both these instances no further treatments were given beyond supplying warmth and a good circulation of air. On March 13, 1906, the writer of this report planted in pots in the greenhouse ordinary potatoes secured from the German market in the city of Halle. They grew and on May 10 young tubers from 1 to 2 cm. in diameter were harvested, cut into pieces and planted. These began to grow on May 27, thus showing that they apparently had no resting period.

In this connection it may be of interest to remark that in the Southern States it is a practice among some of the gardeners to secure their seed potatoes from the North. These grow and mature quickly and are used for seed for a second planting, which is done immediately after harvesting. In this way two crops are annually produced, but it is necessary to procure new Northern seed each year.

The degree of intensity of the rest we have seen varies greatly among the different species, but this is not surprising when we notice the great differences in the other characters. Without an experimental test one cannot know the extent of the dormancy of any particular plant in winter. It may be aroused into growth easily or the rest may be so profound that it can be awakened with great difficulty or not at all, immediately. However, under the most extreme cases of rest one should be cautious in saying that a plant is completely dormant. Even when in a state of winter rest a plant is not necessarily inactive in all respects, for, according to Müller-Thurgau,[21] Simon,[22] and others, respiration continues to go on. In this regard it is interesting to note that Simon, after investigating the subject of the retention of some of the growth activities during the winter rest, gives this conclusion: ". . . . that so far as some of the functions of growth and metabolism and also the respiration are concerned, woody plants have no rest period, for, even in winter, under favorable conditions, these activities reach a relatively high intensity."

In discussing the rest period Johannsen[23] states that it is in the nature of a "hindrance" (Hemmung) which prevents growth, but when growth is secured by treatment, it is because the "hindrance"

[20] Klebs, Georg, Willkürliche Entwickelungsänderungen bei Pflanzen. p. 137.

[21] Müller-Thurgau, H., Landwirtschaftlichen Jahresbericht. Bd. 14 p. 861.

[22] Simon, S., Untersuchungen ueber das Verhalten einiger Wachstumsfunktionen, sowie der Atmungstätigkeit der Laubhölzer während der Ruheperiode. Jahresbericht für wissenschaftliche Botanik. Bd. 43. Heft 1.

[23] Johannsen, W., Das Aetherverfahren beim Frühtreiben mit besonderer Berücksichtigung der Fliedertreiberei. 1906. p. 47.

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is reduced or weakened, or, that the power of growth (Wachstumstätigkeit) is increased. Little is known of the exact nature of the winter rest period but since it appears to be a habit induced by oft-repeated influences, the cause must be sought in the life centers where impressions of any other kind are recorded.

EFFECTS OF ETHER, FREEZING, DRYING, ETC.

The whole question of how vapors and gases, freezing and desiccation, cause growth to begin in dormant plants, or serve as stimulants in plants that are already in growing condition, is as little understood as the rest period itself. According to Drude,[24] Leclerc, more than fifty years ago, was the first to investigate the effects of ether on plants. Since then, however, during the last twenty or twenty-five years, many investigators have tested the effects of ether, as well as other vapors and gases, on plants in connection with the experimental study of respiration, transpiration and movement, but it is only recently—the last five or six years—that they have been used for forcing dormant plants into growth. As early as 1873 and 1874 Heckel[25] studied the behavior of *Berberis* stamens, while they were under the influence of chloroform. Again in 1874[26] he used chloroform, chlorohydrate and nitrous oxide on the stamens of both *Berberis* and *Ruta*, and also during the same year[27] both anesthetics and narcotics were used on many species of plants in the study of sensitiveness and movement. In 1879 Giglioli[28] used ether, etc., in

[24] Drude, O., Naumann, A., und Ledien, F., Ueber die von Ostern 1902 bis 1903 im Botanischen Garten zu Dresden angestellten, den Gartenbau betreffenden Versuche, etc. Jahresber. VII. der "Flora" zu Dresden.

[25] Heckel, E., Des l'irritabilité des étamines, distinction dans ces organes de deux ordres de mouvements. Bull. de la Soc. Botanique de France. t. 20, pp. 280-281.

[26] Heckel, E., Différentiation des mouvements provoqués et spontánés. Etude sur l'action de quelques agents réputés anesthésiques sur l'irritabilité fonctionelles des étamines de *Mahonia*. Comptes rendus LXXVIII, No. 12, pp. 856-859, et Bull. d. l. Soc. Botanique de France. t. 21, pp. 101-103.

[27] Heckel, E., De l'irritabilité fonctionnelle dans les étamines de *Berberis*. Comptes rendus. t. LXXVIII, No. 14, pp. 985-988, et Bull. de la Soc. Botanique de France, t. 21, pp. 95-98.

————, Mouvement provoqué dans les étamines de *Mahonia* et de *Berberis*; conditions anatomiques de ce mouvement. Comptes rendus, t. LXXVIII, No. 16, p. 1162-1164, et Bull. d. l. Soc. Botanique de France, t. 21, pp. 208-210.

————. Du mouvement provoqué dans les étamines des *Syantherées*. Comptes rendus, t. LXXIX, No. 16, pp. 922-925, et Bull. d. l. Soc. Botanique de France, t. 21, pp. 308-311.

————. Du mouvement dans les étamines du *Sparrmannia africana* L. fils, des *Cistes* et des *Heliamthum*. Comptes rendus, t. LXXIX, No. 1, pp. 49-52.

[28] Giglioli, J., Resistenza dei semi, e specialmente dei semi di agenti chimici gassosi e liquidi. Gazetta Chimica Italiana, IX.

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both gaseous and liquid form on dry and wet seeds to test the effects on their germinating powers, and found that all seeds have not the same powers of resistance to the treatment, but that the wet ones were killed much easier than the dry ones. It was not stated whether growth was hastened or not. Ten years later Townsend[29] tested the effects of ether on the germination of seeds and spores. He found that slight ether fumes caused the following to germinate quicker than under normal conditions: Seeds of *Zea Mais, Avena sativa, Phaseolus vulgaris,* and *Cucurbita pepo;* spores of *Mucor* and *Penicillium.* The subject of forcing seeds into growth by the use of gases and vapors and like treatments, however, yet remains uninvestigated except for incomplete, isolated cases. In 1905 Latham[30] found that ether and chloroform hastened the development of spores of *Sterigmatocystis nigra* and *Penicillium glaucum.*

The earlier investigators were much puzzled over the effects of ether on growing plants. In 1879 Arloing[31] caused a *Mimosa* plant to absorb ether and chloroform mixed with water, through a tube. The resulting "intoxication" lasted nearly two hours, during which time all sensitiveness was lost, but returned again with the passing of the effect of the drugs. Macchiati[32] used the same drugs for the same purpose in 1880 and gave it as his opinion that the vaporizing of the materials caused a lowering of the temperature about the plant to such an extent as to prevent movement, but this was disproven by Cugini[33] the next year. Two years later, 1883, Macchiati[34] decided that the anesthetics had the same effect on plants as on animals, that is, that they are absorbed. In 1882 Detmer[35] used chloroform in studying respiration in young pea plants. The treated plants made no advance and finally died off, it being explained that chloroform fully restrained the growth of the cells but that respiration continued vigorously. Tassi[36] in 1884 dipped freshly cut flowers

[29] Townsend, C. O., The effect of ether upon the germination of seeds and spores. Botanical Gazette, XXVII. pp. 456-458.

[30] Latham, Marion Elizabeth, Stimulation of *Sterigmatocystis* by chloroform. Bull. Torrey Bot. Club, 32. pp. 337-351.

[31] Arloing, M., Sur un nouveau mode d'administration de l'éther, du chloroforme et du chloral à la sensitive; application à la détermination de la vitesse des liquides dans les organes de cette plante. Comptes rendus, t. 89. p. 442.

[32] Macchiati, L., Del Movimento periodico spontaneo degli stomi nelli *Ruta bracteosa* DC. e nello *Smyrnium rotundifolium* DC. Nuovo Giorn. Bot. Ital. XII. 3. pp. 243-247.

[33] Cugini, G., Intorno all'azione dell'etere e del cloriformio sugli organi irritabili delle pianti. Nuovo Giorn. Bot. Ital. XIII. 4. pp. 288-291.

[34] Macchiati, L., Ancora sugli anestetici delle piante. Nuovo Giorn. Bot. Ital. XV. 1. pp. 214-221.

[35] Detmer, W., Ueber die Einwirkung verschiedener Gase, insbesondere des Stickstoffoxydulgases auf Pflanzenzellen. Landw. Jahrb. XI, pp. 227, 228.

[36] Tassi, F., Degli effetti anestetici nei fiorie. Nota. Siena.

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of *Croccus, Oxalis,* etc., in solutions of anesthetics and narcotics and noted the results on the opening and closing of the floral parts. The experiments were repeated the following year.[37]

Laurén[38] in 1891 investigated the effects of ether vapor on very young seedlings. Transpiration, as affected by anesthetics, was studied in 1892 by Schneider,[39] and in 1896 by Dixon;[40] also in 1896 Johannsen[41] reported results of work on transpiration and study of the rest period. Sandsten[42] experimented with a number of gases and vapors in regard to their influences on plant growth in 1898. He found that ether dissolved in water accelerated the germination of seeds, and that the fumes of ammonia, chloroform and ether, caused an increased growth in twigs and young plants.

The above review is not intended to be complete, but is only given to show some of the steps in the development of studies on the effects of ether on plant life. In the bibliography list at the end of this bulletin will be found a practically complete list of references to the literature bearing upon the subject of this paper.

Although much work had been done with anesthetics and narcotics on growing vegetables, it was with the appearance of Johannsen's "Aetherverfahren beim frühtreiben mit besonderer Berücksichtigung der Fliedertreiberi," in 1900, that there was aroused a wide-spread interest in the use of ether and chloroform for forcing plants into growth, and since that time there have been a great many references in botanical and gardening periodicals to their practical use.

Regarding the workings of the ether and chloroform Johannsen[43] speculates at length. He does not say that the ether exerts a specific effect on the protoplasm, but from his discussion it is inferred that he believes this to be the case. He declares that the workings of ether and chloroform could be in only two ways, that they act on the "power of growth" (Wachstumstätigkeit), or on the "hindrance" (Hemmung), or possibly on both. This author expressed his disbelief, however, in the theory that the effects of ether,

[37] Tassi, F., Degli effetti anestetici del cloridrato di cocaina sui fiori di alcune piante. Bollettino della Soc. tr. Cult. d. sci. med. d. Siena.

[38] Laurén, W., Om inverkan af eterångor på groddplantors andning.

[39] Schneider, A., Researches on the influence of anesthetics on transpiration. Minn. Acad. Science 17. p. 232.

[40] Dixon, Henry H., On the effects of stimulation and anesthetic gases on transpiration. Preliminary notes. Proc. Royal Irish Acad. III. ser. vol. 4, pp. 618-626.

[41] Johannsen, W., Aether- und Chloroform-Narcose und deren Nachwirkung. Bot. Centralblatt. Bd. 68. pp. 337-338.

[42] Sandsten, E. P., The influences of gases and vapors upon the growth of plants. Minn. Bot. Stud. 2. ser. I. pp. 53-68.

[43] Johannsen, W., Das Aetherverfahren beim Frühtreiben mit besonderer Berücksichtigung der Fliedertreiberei. 1906. pp. 24-60.

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freezing and drying were the same, even though their results are similar. Regarding drying in particular he says, "as a result of my experience it is clear that the effects of desiccation and treating with ether and chloroform are not similar. Only one example will be cited: The seed of the two-row or bearded barley (*Hordeum zeocrithon*) have a pronounced rest period. Drying has no effect at all, while a very slight amount of ether causes them to start into growth in the shortest possible time."[44] He also disagrees with Dubois[45] who reported that ether exerts a strong dehydrating effect and is therefore similar to freezing and drying.

In the writer's experiments it was often noticed that there was considerable moisture in the glass jars after etherizing a big bundle of twigs for forty-eight hours or longer. Whether this moisture resulted from the ordinary transpiration, which was thought to be the case, or had been extracted by the ether, is not clear. The quantity of water in the jar was especially noticeable following the etherization of twigs that had been for several days in the dark-chamber. These facts are significant when taken in connection with the experiments of Jumelle,[46] who found in 1890 that ether caused an increase of transpiration in the light and a decrease in darkness. There was no ether in the dark-chamber in the writer's experiments, but the air there was saturated with moisture, and hence transpiration was reduced to a minimum, so that conditions were favorable, when they were removed and placed in the jar, for the ether to cause the plants to transpire to their utmost, in accordance with Jumelle's findings.

It has also been found that ether increases the respiration, but this in itself does not constitute growth. Müller-Thurgau[47] has shown that growth is preceded by sugar being made available as fuel for the greatly increased respiration and that this sugar comes from a reserve supply as such, or is formed from starch or other carbohydrates present, through the agency of diastase of ferment. He further found that sugar is formed very rapidly in potatoes at o degrees C. At this temperature, it is explained, the protoplasmic activities are much depressed and hence the fermentation processes produce more sugar than is used, and it accumulates. But at higher temperatures respiration and the formation of starch rapidly increase

[44] Johannsen, W., Das Aetherverfahren beim Frühtreiben mit besonderer Berücksichtigung der Fliedertreiberei. 1906, p. 57.

[45] Dubois, R., Mécanisme de l'action des anésthésiques. Rev. gen. d. Sci. pures et appliqués, 2. an. No. 17. pp. 261-567.

[46] Jumelle, H., Influence des anesthésiques sur la transpiration des végétaux. Rev. gen. de Bot. 2. pp. 417-432.

[47] Müller-Thurgau, H., Ueber Zuckeranhäufung in Pflanzenteilen infolge niederer Temperatur. Landw. Jahresber. 11. pp. 751-828.

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and at 10 degrees the protoplasm needs nearly all of the sugar that has been formed and at 20 degrees all the newly formed sugar—as well as any reserve supply that might have been there—is consumed, and more would be if it were available. After holding potatoes at 0 degrees for a long time and then removing them to a warm room, it was found that the respiration during the first week was much greater than normal, which he believed to be the indirect result of the large amount of sugar that was placed at the disposal of the protoplasm, and not through any direct stimulus the protoplasm had received from the freezing.

The foundation principle to be worked from is whether a given agency, as ether, drying, etc., will cause the formation of sugar in a plant, and as to whether this has been done can only be decided by quantitative chemical determinations. But will ether cause any such results? It is of interest here that Latham[48] found that in chloroformed fungi the increased growth was accompanied by less acid formation and less sugar consumption, thus indicating increased economy in metabolism. Gerrassimow[49] studied the effects of ether on *Spirogyra* and concluded that the increased growth was caused by the anesthetic having stimulated the cell nucleus.

Just here it will be appropriate to notice the results of the experiments with the young oaks growing in pots, previously mentioned (see pages 12 and 80). In this case there were two agencies that acted on the same plant, both producing an increased growth. These agencies were freezing and ether, but it was undoubtedly the former that caused growth to begin, although the extent of the freezing was very slight. Since freezing and ether affect plants similarly, it seems reasonable to conclude that the latter does not exert any specific chemical action on the protoplasm, which may somewhat narrow the question, but by no means answers it.

It is popularly supposed that drying is very similar to freezing, as both remove water from the plant body and the latter at least, quickly condenses the cell sap. They may, however, not be identical in their effects. Freezing, according to Molisch,[50] Mez,[51] and Pfeffer,[52] forces the moisture out of the cells in the form of chemically pure water, leaving all of the salts behind. But these processes

[48] Latham, Marion Elizabeth, Stimulation of *Sterigmatocystis* by chloroform. Bull. Torrey. Bot. Club, 32. pp. 337-351.

[49] Gerrassimow, J., Aether-Kulturen von *Spirogyra*. Flora. Bd. XCIV. pp. 79-88.

[50] Molisch, Hans, Untersuchung ueber das Erfrieren der Pflanzen. pp. 16-26.

[51] Mez, Carl, Neue Untersuchungen ueber das Erfrieren eisbeständiger Pflanzen. Flora oder Allg. bot. Zeitung. Bd. 94. Heft 1. p. 94.

[52] Pfeffer, W., Pflanzenphysiologie. Bd. II. 1904. p. 308.

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need not necessarily injure the cells, merely condensing the cell sap, while the process of drying leaves different effects. Schröder [53] found that severely dried cells were broken and that the protoplasmic bodies were thickened ("trübe"), opaque, and appeared denser and darker than in turgescent cells. He also noted the presence of air in the cells.

Aymard [54] gave it as his opinion that anesthetics produce the same effects on vegetation as freezing or drought; that they act on vegetable tissue as dehydrating agents. As proof of this, he found that roots of the lily-of-the-valley placed under a bell-jar with phosphoric anhydrid and other drying liquids, forced even more quickly than when submitted to ether vapor. It was also found that etherized plants lose considerable in weight.

A dark-chamber, which is also very moist, may exert an effect in causing growth, in two ways. The beneficial effects of a uniform temperature of 20 degrees or 22 degrees, combined with a high humidity, are too well known to need comment. At the high temperature of 22 degrees C. respiration would be very vigorous and sugar rapidly consumed, but under the favoring influence of the darkness [55] and warmth, the ferments would be very active and sugar could be formed in greater quantities than normally. This may account for the large percentage of the twigs that grew in the dark-chamber, but there is no explanation for the slowness with which this growth took place, a feature which is so prominent in the experimental data.

In conclusion it may be added that whether etherizing, drying and freezing are alike or related in the nature of their workings or not, the experimental results herein recorded show that all will break the winter resting period of plants. Etherizing has been referred to as "stimulating" or "poisoning" the plants, the freezing and drying as concentrating the cell sap and therefore also as "stimulants," but all of these terms bring us no nearer to an answer to the question. It is very probable, however, that none of the agents used for forcing growth exert any specific action, but are rather the means, possibly in distinctly different ways, of setting into activity, indirectly, certain complicated vital machinery which results in growth.

[53] Schröder, G., Ueber die Austrocknungsfähigkeit der Pflanzen. Tübinger untersuchungen. Bd. II.

[54] Aymard fils, J., Les anesthésiques et le forcage des plantes.

[55] Green, J. R., Influence of light on diastase. An. of Bot. Vol. VIII, pp. 561-567.

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LITERATURE.

The literature mostly used during the course of the investigation was as follows: Johannsen's well-known "Aetherverfahren beim Frühtreiben, etc.," editions of 1901 and 1906, which served as a basis for some of the mechanical operations, for example, the quantity of ether to use and the length of time for it to act, as well as for a number of other useful suggestions. In the discussion, Klebs' valuable books "Willkürliche Entwickelungsänderungen bei Pflanzen," Chap. VI, and "Ueber Variationen der Blüten," Abschnitt IV, as well as Pfeffer's "Pflanzenphysiologie," Zweiter Band, 1904, were used. Occasional references throughout this report indicate other sources consulted, but there are a few authors mentioned in the list of literature at the end of this paper that are not specifically cited in the text, although they were drawn upon in the general study.

OUTLINE OF EXPERIMENTS.

The preliminary steps in the experimental study of the rest period in plants, was to find what species would grow by merely being brought into a warm, moist room, and those not so growing to be treated by different methods until growth was secured. The following outline was followed as nearly as possible:

1. *Effects of Warmth Alone.*

All of the species were placed in the greenhouse in late fall to see which would grow without treatment. For purposes of comparison the same list was again taken into the warm room in midwinter. Those that made no growth were again collected in late winter; and a few that still refused to grow were brought in just before the beginning of spring. See Tables I and II—283 species.

2. *Treatment with Ether Only.*

A number of the species that would not grow in the greenhouse and some that did, were treated with ether only. See Table III—58 species.

3. *Freezing, Etherizing and Use of Dark-Chamber.*

A list of kinds that grew with difficulty or not at all, without treatment, were treated by a few methods to force growth; viz., freezing; freezing and then etherizing; and, enclosing for a time in a moist, dark-chamber. See Table VI—35 species.

4.—*Etherizing, Drying, Freezing, and Dark-Chamber, Singly and in Combination.*

A longer list of sorts that, for the most part, were known to be difficult to force into growth, were treated as follows: with ether,

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by drying, by freezing, enclosing in moist dark-chamber, and with combinations of freezing, etherizing and the use of the dark-chamber. See Table IX—70 species.

5. *Miscellaneous and Special Tests.*

These tests for the most part employed ether and drying, but also in addition the use of chloroform both as a liquid and as a gas; ether as a liquid, desiccation with alcohol, with salt solution, and with sulphuric acid; injecting with sugar and water solutions under pressure; moist light-chamber; and by wounding the buds. Treatment of a few oaks growing in pots; also test of a short list of species of Mediterranean plants in the greenhouse without treatment.

GENERAL NOTES ON METHODS AND CONDITIONS OF THE EXPERIMENTS.

It was not possible to secure potted plants, so small branches from 30 to 60 centimeters long were used, each being securely labeled and kept standing in water when in the greenhouse or dark-chamber. After each treatment plants were immediately transferred to the greenhouse, where they remained until they either grew or died. The day temperature of the greenhouse throughout the winter varied between 20° and 22° and at night from 15° to 17° C. The relative humidity ranged between 50 and 80 %.

Etherizing.

The quantity of ether used for each application was 40 grams per 100 litres of space in the vessel, regardless of the number of plants being treated at one time. The twigs were placed in cylindrical glass jars about one meter high and 20 centimeters in diameter. The tops were closed with ground glass plates having a small opening in the center to admit the ether. The plates were smeared with a soft wax and then pressed firmly down upon the rim of the jars. The ether was poured through a small funnel, which was quickly removed and the hole securely closed with a cork stopper. The covers were then held down by placing upon them jars of sand weighing 8 to 10 kilos. This precaution was necessary, as there was considerable pressure from the ether vapor. The temperature was usually between 18 and 20 C.

Freezing.

The plants were frozen in a specially prepared refrigerator. This was a wooden box about one meter high and containing approximately two hectoliters of space. The walls were thick and lined with zinc. Then there was a smaller, square-cornered vessel made of zinc which fitted inside of the refrigerator in such manner as to leave a space five or six cm. wide between the walls of the two boxes, all

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around. A mixture of common salt and crushed ice was packed in the bottom of the outer box to a depth of about 10 cm., then the zinc vessel was fitted into place and the space between the walls packed with the freezing mixture. Every day the water from the melting ice was drawn off and the supply of the freezing material replenished.

The refrigerator was kept all the time in a cool basement room. The plants were placed in the inner, metal box, and a thermometer thrust in among them. Daily readings showed that the temperature varied between -5 and -10 C., depending upon the changes of the atmospheric temperature outside. For the most part the plants were exposed to a temperature of -6 to -10. After a day or two the twigs became quite brittle from the cold. Potatoes and onions placed in the box were frozen perfectly rigid in a few hours. The twigs were thawed out gradually before being removed to the warm room for further treatments.

Dark-Chamber.

The dark-chamber used was made of glass with thoroughly darkened walls, and was about one cubic meter in size. Doors at one side admitted the plants. It rested on top of a laboratory case and in this position at the top of the room the temperature was not only somewhat higher but was more uniform than below. The temperature at a midpoint in this laboratory room varied between 18° and 20°, while in the chamber it remained almost constantly around 22°. In the chamber the twigs stood in a large vessel of water and thus the atmosphere was kept moist. The hygrometer showed the humidity to be from 90 to 92%.

Desiccation.

Except in a few special cases the twigs were air-dried in a warm, dry, laboratory room. The temperature of the room varied from 18 to 20°, and the humidity from 30 to 35%. The twigs were first carefully weighed to the tenth of a gram and then spread out on a table. At a given time each day a portion was removed, again accurately weighed, and one or two centimeters clipped from the lower ends, as the freshly cut parts soon shriveled from drying. Those dried two days or more and appearing very dry, were submerged in water for about an hour before being placed with the others in the greenhouse.

The Dates.

To make the tables as simple as possible, only two dates are given after each species. These dates are, first, the number of days that elapse from the time of treatment until the first growth was noticed, and, second, the number of days from the time of treatment

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until the first buds (leaves or flowers) were fully open. In all cases these dates were reckoned from the time the plants were placed under growing conditions. In most instances the starting point was when placed in the greenhouse. An exception, however, was when the plants were treated in the dark-chamber. Here they had both warmth and moisture, and hence dates were computed from the time they were placed inside.

Controls.

For each lot of twigs treated there was at least one specimen from every species left untreated to check the results. In the greenhouse, controls and all were kept under exactly similar conditions.

EXPERIMENTAL DATA, 1905-6.

1. *A Test of Effects of Warmth Alone* (Tables I and II).

From October 28 to November 4, 1905, a set of twigs was collected representing 234 species. These were placed in the greenhouse untreated, as this was a preliminary test to find which would grow merely under the influence of warmth.

About ten weeks later—January 8 to 10, 1906, another set was collected including the 234 kinds of the first collection and 49 additional species, making a total of 283. These were placed in the greenhouse without treatment as before, to compare effects of season on the growth. In Table I are the names of all of the species of the two collections mentioned above, with the native country of each, the number of days required for growth to begin and for the buds to open fully. The figures refer to number of days from time plants were placed in the greenhouse. The characters "." and "*" indicate respectively whether the growth was from a leaf bud or from a flower bud.

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TABLE I.

Twigs brought into the greenhouse without previous treatment.

. = Leafbud. * = Flowerbud. 0 = No growth.

SPECIES.	HABITAT.	Collected Oct. 20-Nov. 4, 1905.		Collected Jan. 8-10, 1906.	
		Growth began in	Buds fully open in	Growth began in	Buds fully open in
		Days	Days	Days	Days
Acanthopanax pentaphyllum Marsh	China & Japan	. 28	. 34	. 8	. 7
Acer campestre L.	Europe & Orient	0	0	. 17	. 25
Acer campestre L., var. hebecarpum	Hort. form	. 41	0	. 18	0
Acer campestre L., var pulverulentum	Hort. form	0	0	. 26	0
Acer dasycarpum Ehr.	N. Amer.	0	0	. 39	. 45
Acer Ginnala Maxim	N. China & Japan	. 13	. 19	. 2	. 9
Acer macrophyllum Pursh	N. Amer.	0	0	* 31	0
Acer monspessulanum L.	S. Europe	0	0	. 12	* 20
Acer negundo L.	N. Amer.	0	0	. 25	. 34
Acer negundo L., var. californicum	California	0	0	. 38	. 45
Acer negundo L., var. crispum	N. Amer.	....	....	0	0
Acer negundo L., var. fol., arg. marg.	Hort. form	....	....	. 31	. 41
Acer negundo L., var. fol. aur. marg.	Hort. form	....	....	. 39	. 45
Acer negundo L., var. vernicolor	N. Amer.	. 63	. 69	. 20	. 26
Acer negundo L., var. violaceum	N. Amer.	. 64	. 73	. 24	. 35
Acer platanoides L.	Europe	0	0	. 13	* 20
Acer Pseudo-Platanus L.	Europe	0	0	0	0
Acer spicatum L.	N. Amer.	0	0	0	0
Acer tataricum L.	E. & S. E. Europe	0	0	. 8	* 10
Acer tauricum	Europe & Orient	0	0	. 10	. 21
Aesculus chinensis Bunge	China	0	0	. 33	. 45
Aesculus flava Ait	N. Amer.	....	....	. 35	0
Aesculus Hippocastanum L.	Orient	0	0	. 20	. 25
Aesculus humilis Lindl.	N. Amer.	....	....	0	0
Aesculus macrostachya Mchx.	N. Amer.	....	....	. 25	. 45
Ailanthus glandulosa Dsf.	China & Japan	....	....	. 6	. 16
Alnus americana C. Koch.	N. Amer.	0	* 49	. 15	. 20
Alnus barbata C. A. Mey.	Caucasus	0	* 27	. 16	. 22
Alnus glutinosa Medic.	Europe, Asia, Afr. & Amer.	0	0	. 13	. 21
Alnus japonica S. & Z.	Japan	0	0	. 10	. 18
Alnus viridis DC.	Europe, Asia, & N. Amer.	....	....	0	0
Amelanchier canadensis Medic.	N. Amer.	0	0	. 5	. 22
Amelanchier ovalis Lindl.	N. Amer.	0	0	. 4	* 7
Amelanchier vulgaris Moench.	S. Europe	. 44	0	. 16	* 22
Amorpha canescens Nutt.	N. Amer.	. 38	. 50	0	0
Amorpha fruticosa L.	N. Amer.	0	0	0	0
Amygdalus communis L.	Orient	* 15	* 25	. 2	* 8
Amygdalus Gaertneriana Schlect.	Europe & Asia	. 13	. 16	. 2	* 9

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TABLE I. (*Continued.*)

Twigs brought into the greenhouse without previous treatment.

. = Leafbud. * = Flowerbud. 0 = No growth.

SPECIES.	HABITAT.	Collected Oct. 20-Nov. 4, 1905.		Collected Jan. 8-10, 1906.	
		Growth began in	Buds fully open in	Growth began in	Buds fully
		Days	Days	Days	Da
Amygdalus nana L.	Asia Minor	....	....	. 8	*
Amygdalus persicoides C. Koch	Europe	....	....	. 2	*
Andromeda calyculata L.	Europe, Asia & N. Amer...	0	0	. 9	.
Andromeda japonica Thunbg.	Japan	0	0	. 8	.
Andromeda mariana L.	N. Amer.	. 9	. 17	. 5	.
Andromeda paniculata L.	N. Amer.	0	0	. 8	.
Andromeda speciosa Michx.	N. Amer.	. 12	. 0	* 8	.
Azalea mollis Blume	China & Japan	. 26	. 45	0	
Azalea pontica L.	Asia Minor	* 48	* 53	* 17	*
Benzoin odoriferum Nas.	N. Amer.	0	0	0	
Berberis vulgaris L.	Europe & Asia	. 10	. 18	. 8	.
Betula alba L.	Europe & Asia	. 25	* 27	* 7	*
Betula nana L.	Europe, Asia & N. Amer...	....	....	. 2	
Betula nigra L.	N. Amer.	. 62	. 78	. 12	.
Broussonetia papyrifera Vt.	China & Japan	. 32	. 57	. 6	*
Buddley intermedia H. B.	Mexico	. 2	. 5	. 4	.
Calophaca wolgarica Fisch.	S. Russia	. 9	. 17	. 6	.
Calycanthus floridus L.	N. & S. Caro.	....	....	. 21	
Calycanthus occidentalis Hoot. & Arn.	California	. 9	. 16	. 6	.
Caragana arborescens Lam.	Siberia	0	0	. 8	.
Caragana arborescens Lam. var. pendula	Hort. form	. 9	. 15	. 2	.
Caragana spinosa DC.	Siberia	6	. 13	. 2	.
Carpinus americana Michx.	N. Amer.	0	0	. 34	.
Carpinus betulus L.	Europe	. 8	0	. 14	.
Carya aquatica Nutt.	N. Amer.	0	0	0	
Carya porcina Nutt.	N. Amer.	0	0	0	
Castanea pumila Michx.	N. Amer.	0	0	. 17	
Castanea vesca Grtn.	S. Europe	0	0	0	
Catalpa Kaempferi S. & Z.	Japan	. 16	. 22	. 11	.
Catalpa syringifolia Sims.	N. Amer.	. 17	. 24	. 5	.
Cedrela sinensis Juss.	China	0	0	. 9	.
Celastrus scandens L.	N. Amer.	0	0	. 17	.
Celtis occidentalis L.	N. Amer.	. 33	. 37	* 1	
Celtis Tournefortii Lam.	Orient	0	0	. 10	.
Cercidiphyllum japonicum S. & Z.	Japan	0	0	. 21	.
Cercis canadensis L.	N. Amer.	0	0	. 10	
Cercis Siliquastrum L.	Europe & Orient	0	0	. 21	
Chionanthus fragrans Lindl.	N. Amer.	0	0	. 9	

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TABLE I. (*Continued.*)

Twigs brought into the greenhouse without previous treatment.

. = Leafbud. * = Flowerbud. 0 = No growth.

SPECIES.	HABITAT.	Collected Oct. 20-Nov. 4, 1905.		Collected Jan. 8-10, 1906.	
		Growth began in	Buds fully open in	Growth began in	Buds fully open in
		Days	Days	Days	Days
Clethra acuminata Michx.	N. Amer.	0	0	0	0
Clethra alnifolia L.	N. Amer.	0	0	9	. 13
Clethra barbinervis S. & Z.	Japan	0	0	. 6	. 9
Colutea arborescens L.	Europe & Orient	0	0	. 10	0
Colutea cruenta Ait.	S. Europe & Orient	. 8	. 24	. 4	. 7
Comptonia asplenifolia Banks	N. Amer.	. 26	. 28	. 6	. 9
Cornus alba L.	Siberia	0	0	. 7	. 10
Cornus candidissima Mill.	N. Amer.	0	0	. 10	. 14
Cornus Mas L.	Middle & S. Europe	. 18	. 21	* 3	* 5
Cornus sanguinea L.	Europe & Orient	0	0	. 11	. 13
Corylus Avellana L.	Europe & Asia	0	0	* 4	. 22
Corylus Colurna L.	Asia Minor & Turkey	0	* 25	* 6	. 21
Crimeasier vulgaris Ldl.	Europe & Orient	0	0	. 4	. 10
Crataegus betulaefolia	Hort. form	. 7	. 25	. 3	. 9
Crataegus chinensis Steud.	China	. 11	. 22	. 3	. 9
Crataegus coccinea L.	N. Amer.	0	0	. 3	. 21
Crataegus flava Ait.	N. Amer.	0	0	. 6	. 10
Crataegus floribunda C. Koch	N. Amer.	0	0	. 16	. 26
Crataegus latifolia L.	N. Amer.	0	0	. 16	0
Crataegus macracantha Lodd.	N. Amer.	0	0	. 21	0
Crataegus melanocarpa M. B.	Caucasus	. 20	. 44	. 6	. 15
Crataegus nigra W. & K.	Hungary	* 6	. 18	. 3	. 8
Crataegus oliveriana B. & DC.	Europe	. 15	. 28	. 5	. 26
Crataegus Oxyacantha L.	Europe	. 15	. 36	. 3	. 7
Crataegus Oxy. L., var. fl. rubr. pl.	Hort. form	. 5	. 15	. 3	. 10
Crataegus Oxy. L., var. fol. tricolor	Hort. form	. 12	. 35	. 4	. 12
Crataegus punctata, var. aurea Jacq.	N. Amer.	0	0	. 17	. 26
Crataegus spinosissima Lodd.	Siberia	0	0	. 7	. 11
Crataegus stipulacae	Europe	0	0	. 21	. 36
Crataegus tanacetifolia Per.	Asia Minor	0	0	. 18	. 39
Cydonia japonica Pers.	Japan	* 6	* 16	. 3	. 7
Cydonia vulgaris L.	S. Europe	. 6	. 15	. 7	. 11
Cytisus laburnum L.	Europe	....	....	. 9	. 0
Cytisus weldeni Vis.	S. Hungary	* 5	* 21	. 3	0
Deutzia crenata S. & Z.	Japan	. 9	. 16	. 4	. 9
Deutzia gracilis S. & Z.	Japan	. 10	. 21	. 3	* 17
Deutzia scabra Thb.	Japan	....	....	. 4	. 9
Dirvilla canadensis Willd.	N. Amer.	0	0	0	0

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TABLE I. (*Continued.*)

Twigs brought into the greenhouse without previous treatment.

. = Leafbud. * = Flowerbud. 0 = No growth.

SPECIES.	HABITAT.	Collected Oct. 20-Nov. 4, 1905.		Collected Jan. 8-10, 1906.	
		Growth began in	Buds fully open in	Growth began in	Buds fully open in
		Days	Days	Days	Day
Diospyros virginiana L.	N. Amer.	....	....	0	
Elaeagnus argentea Pursh.	N. Amer.	0	0	. 7	
Erica carnea L.	S. Europe	0	0	. 20	
Euomymus alatus Rupr.	China & Japan	0	0	. 5	. 2
Euonymus americanus L.	N. Amer.	....	....	. 20	
Euonymus europaeus L.	Europe & Orient	0	0	. 8	. 1
Fagus sylvatica L.	Europe & W. Asia	0	0	. 38	
Fagus sylvatica L., var. atropurpurea	Europe	....	....	. 36	. 3
Forsythia suspensa Vahl.	China & Japan	* 7	* 14	* 8	*
Fraxinus americana L.	N. Amer.	....	....	0	
Fraxinus excelsior L.	Europe & W. Asia	....	....	0	
Fraxinus excelsior, var. hetrophylla, L.	Asia	....	....	. 33	. 3
Fraxinus oregona Nutt.	N. Amer.	....	....	0	
Fraxinus Ornus L.	S. Europe	0	0	0	
Ginkgo biloba L.	Japan	0	0	. 19	. 2
Gleditschia triacanthos L.	N. Amer.	0	0	. 5	. 1
Gymnocladus canadensis Lam.	N. Amer.	....	....	. 5	. 1
Halesia tetraptera L.	N. Amer.	0	0	. 10	. 3
Halimodendron argenteum Fisch.	Siberia & Tartary	. 9	. 14	. 7	. 1
Hamamelis virginiana L.	N. Amer.	0	0	0	
Hippophae rhamnoides L.	Middle Europe & N. Asia	. 6	. 11	. 4	. 1
Hydrangea radiata Walt.	N. Amer.	....	....	0	
Itea virginica L.	N. Amer.	0	0	. 7	. 1
Jasminium fruticans L.	S. Europe, N. Afr. & Orient	....	....	. 10	
Juglans cinerea L.	N. Amer.	....	....	. 22	. 2
Juglans nigra L.	N. Amer.	. 60	. 65	. 22	. 2
Juglans regia L.	Europe & Asia	0	0	0	
Kalmia angustifolia L.	N. Amer.	0	0	0	
Koelreuteria paniculata Laxm.	China	0	0	. 9	
Ledum latifolium Jacq.	N. Amer.	0	0	0	
Ledum palustre L.	Europe, Asia & N. Amer.	....	....	. 9	*
Ligustrum vulgare L.	Europe	. 7	. 19	. 8	.
Liquidambar styraciflua L.	N. Amer.	0	0	. 38	.
Liriodendron tulipifera L.	N. Amer.	0	0	0	
Lonicera alpigena L.	Europe	. 4	. 17	. 13	.
Lonicera Ledebourii Esch.	California	. 10	. 63	. 5	.
Lonicera nigra L.	Europe & Asia	. 5	. 26	. 6	.
Lonicera tatarica L.	Siberia	. 7	. 14	. 8	.

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TABLE I. (*Continued.*)

Twigs brought into the greenhouse without previous treatment.

. = Leafbud. * = Flowerbud. 0 = No growth.

SPECIES.	HABITAT.	Collected Oct. 20-Nov. 4, 1905.		Collected Jan. 8-10, 1906.	
		Growth began in	Buds fully open in	Growth began in	Buds fully open in
		Days	Days	Days	Days
Lonicera villosa DC.	S. Hungary	0	0	. 4	. 8
Lycium barbarum L.	Europe & Orient	. 8	. 12	. 2	. 8
Maclura tinctora Don. & Steud.	N. Amer.	0	0	. 6	. 11
Magnolia acuminati L.	N. Amer.	. 29	. 43	* 25	. 36
Magnolia Soulangeana Hort.	Japan	* 11	* 25	* 9	0
Mespilus germanica L.	Europe	. 14	. 64	. 10	. 17
Morus alba L.	Asia Minor & W. Asia	0	0	. 7	. 14
Myrica cerifera L.	N. Amer.	. 26	. 38	. 5	. 18
Myrica Gale L.	Europe & N. Amer.	0	0	. 6	* 9
Nuttalia cerasiformis T. & Gr.	N. Amer.	0	0	. 1	. 6
Ostrya vulgaris Willd.	S. Europe & Orient	0	0	. 32	. 0
Philadelphus Gordonianus Lindl.	California	0	0	. 11	. 21
Photinia villosa DC.	Japan	....	....	. 12	. 19
Platanus orientalis L.	Europe & Orient	. 23	. 31	. 7	. 20
Populus balsamifera Lyall	California	....	....	. 13	. 19
Populus canadensis Foug.	N. Amer.	. 61	. 68	. 20	. 24
Populus candicans Ait.	Asia & N. Amer.	. 32	. 36	. 16	. 19
Populus monilifera Rosier	N. Amer.	. 63	. 68	. 19	. 25
Populus pyramidalis Ait.	S. Europe	. 31	. 37	. 8	. 13
Prunus acida Dum.	Europe	* 17	. 23	. 6	. 19
Prunus Armeniaca L.	Caucasus	....	....	. 4	. 14
Prunus avium L.	Europe & Orient	. 5	. 22	. 4	* 6
Prunus cerasus L.	Asia Minor	* 13	* 21	. 6	. 19
Prunus domestica L.	Caucasus	. 23	. 32	. 6	* 15
Prunus mahaleb L.	S. Europe & Orient	. 60	0	. 12	. 18
Prunus padus L.	Europe & Asia	0	0	. 7	. 12
Prunus Persica Stokes	Asia	0	0	* 6	0
Prunus Pissardi Carr.	Orient	....	....	. 3	. 13
Prunus serotina Ehr.	N. Amer.	0	0	* 5	. 14
Prunus triloba Lindl.	China	* 12	* 25	* 4	* 8
Prunus virginiana L.	N. Amer.	* 51	0	. 7	. 20
Ptelea trifoliata L.	N. Amer.	0	0	0	0
Pterocarya caucasica C. A. Mey	Orient	....	....	. 5	. 8
Pyrus americana DC.	N. Amer.	0	0	. 17	. 20
Pyrus Aria Ehrh.	Europe & Asia	....	....	. 22	. 27
Pyrus Aria, var. edulis Ehr.	Europe & Asia	0	0	0	0
Pyrus aucuparia Ehrh	Europe & Asia	. 40	. 52	. 5	. 10
Pyrus cerasifera Tausch.	Siberia	. 8	. 13	. 4	. 9

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TABLE I. (*Continued.*)

Twigs brought into the greenhouse without previous treatment.

. = Leafbud. * = Flowerbud. 0 = No growth.

SPECIES.	HABITAT.	Collected Oct. 20-Nov. 4, 1905.		Collected Jan. 8-10, 1906.	
		Growth began in	Buds fully open in	Growth began in	Buds fully open in
		Days	Days	Days	Days
Pyrus communis L.	Europe & Asia	. 15	. 26	* 3	. 9
Pyrus graeca Hort.	Europe & Asia	0	0	. 12	0
Pyrus malus L.	Europe & Asia	. 22	. 29	. 5	. 13
Pyrus microcarpa DC.	N. Amer.	. 26	. 32	. 7	. 13
Pyrus nivalis Jacq.	Europe & Asia	....	....	. 6	. 14
Pyrus Polveria L.	Europe	....	....	. 7	. 20
Pyrus prunifolia Willd.	Siberia	. 4	. 11	. 5	. 9
Quercus alba L.	N. Amer.	0	0	0	0
Quercus ambigua W.	N. Amer.	. 26	. 39	0	0
Quercus Cerris L.	S. Europe & Asia	....	....	0	0
Quercus coccinea Wang.	N. America	0	0	0	0
Quercus Dshorochensis C. Koch.	Europe	0	0	0	0
Quercus imbricaria Michx.	N. America	0	0	. 6	0
Quercus macranthera F. & M.	Caucasus	0	0	0	0
Quercus macrocarpa Michx.	N. America	. 59	* 63	0	0
Quercus olivaeformis Michx.	N. America	0	0	0	0
Quercus pedunculata W.	Europe N. Africa & Orient.	. 30	. 40	. 19	. 29
Quercus pedunculata, var. fastigiata W.	S. Europe	....	....	. 6	. 21
Quercus pubescens W.	Europe & W. Asia	. 20	0	. 18	. 21
Quercus ramosa Booth	N. America	. 49	. 61	. 24	. 31
Quercus rubra L.	N. America	0	0	. 17	. 21
Quercus tomentosa Michx.	N. America	0	0	. 26	. 35
Rammus Pallasii Fisch. & Mey	Europe & Asia	. 20	. 23	. 10	. 15
Rhamnus frangula L.	Europe & Siberia	. 21	. 25	. 10	. 14
Rhamnus grandifolia F. & M.	Caucasus & Persia	. 11	. 29	. 12	. 19
Rhododendron ponticum var. Cunninghami L.	S. Europe & Asia Minor	0	0	0	0
Rhodora canadensis L.	N. America	* 28	* 32	. 8	. 26
Rhus Toxicodendron L.	N. America	....	....	* 17	* 30
Rhus typhina L.	N. America	....	....	. 5	. 10
Rhus vernicifera DC.	Japan	....	....	. 11	. 16
Rhus viridiflora Poir.	N. America.	....	....	. 4	. 9
Ribes aureum Pursh.	N. America	. 22	. 46	. 14	. 17
Ribes multiflor W. & K.		. 11	0	. 5	. 14
Ribes nigrum L.	Europe and Asia	. 16	0	. 6	. 15
Ribes rubrum L.	Europe, Asia & America	. 14	0	. 7	. 16
Ribes sanguineum Pursh.	N. America	....	....	. 4	. 18
Robinia Pseud-acacia L.	N. America	....	....	. 13	0
Robinia Pseud-acacia var. amorphaefolia L.	N. America	....	....	. 9	. 13

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TABLE I. (*Continued.*)

Twigs brought into the greenhouse without previous treatment.

. = Leafbud. * = Flowerbud. 0 = No growth.

SPECIES.	HABITAT.	Collected Oct. 20-Nov. 4, 1905.		Collected Jan. 8-10, 1906.	
		Growth began in	Buds fully open in	Growth began in	Buds fully open in
		Days	Days	Days	Days
Robinia Pseud-acacia var. semperflorens L. ..	N. America	. 56	. 68	0	0
Robinia viscosa Vent.	N. America	. 18	. 25	. 11	. 15
Rosa aromatica multiflor Thunb.	Japan	. 4	. 9	. 4	. 5
Rosa canina L.	Europe & Asia	. 8	. 13	. 2	. 6
Rosa Gmelini Bunge	Siberia	. 7	. 14	. 4	. 7
Rosa rubrifolia Vill.	Europe	. 15	. 28	. 7	. 14
Rosa rugosa Thnbg.	Japan	. 8	. 11	. 5	. 7
Rubus Idaeus L.	Europe & Orient	. 9	. 23	. 9	. 15
Salisburia adiantifolia Sm.	China & Japan	0	0	. 24	0
Salix acuminata Lange.	Europe	0	0	. 5	* 10
Salix alba L.	E. Asia & N. Africa	. 9	. 24	. 9	. 12
Salix alba argentea Wimm.	Europe, Asia & N. Africa ..	. 8	. 22	. 10	. 13
Salix alba, var. vitellina L.	E. Asia & N. Africa	....	....	. 9	. 15
Salix alba vitellina pendula L.	E. Asia & N. Africa	. 7	. 11	. 5	. 6
Salix argentea Smith	Europe & Siberia	....	....	* 2	. 0
Salix daphnoides Vill.	Europe & Asia	. 18	. 24	* 5	* 6
Salix laurina Sm.	Europe	. 7	. 27	. 10	. 15
Salix mollissima Ehrh.	Europe	. 7	. 14	. 5	. 8
Salix pentandra L.	Europe & Asia	0	0	* 5	* 10
Salix viminalis L.	Europe & Asia	0	0	. 7	. 11
Salix vitellina L.	Europe, Asia & N. America.	. 9	. 14	. 5	. 8
Sambucus nigra L.	Europe, Orient & Ural	. 8	. 7	. 2	. 3
Sophora Japonica L.	Japan & China	0	0	0	0
Sorbus graeca Lodd. & Steud.	Europe & Asia	0	0	. 15	. 20
Spiraea Lindleyana Wall.	Asia	. 8	. 24	. 7	. 13
Spiraea sorbifolia L.	Asia	. 1	. 5	. 1	. 8
Staphylea pinnati L.	S. & E. Europe	0	0	* 11	0
Staphylea trifolia L.	N. America	....	....	. 4	. 14
Symphoricarpus racemosus Michx.	N. America	. 4	. 40	. 6	. 14
Syringa chinensis Willd.	China	. 10	. 14	. 5	. 8
Syringa emodi Wall.	Himalaya	. 37	. 44	. 14	. 22
Syringa Josikaea Jacq.	Hungary	. 22	. 33	. 12	. 20
Syringa vulgaris L.	Orient	. 9	. 56	. 4	. 7
Tamarix gallica L.	Europe, Asia, Africa	. 61	. 66	* 9	. 20
Tilia alba Ait	N. America	....	....	. 21	0
Tilia argentea DC.	Europe	. 24	. 35	. 10	0
Tilia euchlora C. Koch.	Orient	....	....	. 18	0
Tilia grandifolia Ehr.....	Europe	0	0	. 18	. 21

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TABLE I. (*Continued.*)

Twigs brought into the greenhouse without previous treatment.

. = Leafbud. * = Flowerbud. 0 = No growth.

SPECIES.	HABITAT.	Collected Oct. 20-Nov. 4, 1905.		Collected Jan. 8-10, 1906.	
		Growth began in	Buds fully open in	Growth began in	Buds fully open in
		Days	Days	Days	Days
Tilia handwort	Siberia	....	....	. 25	0
Tilia parvifolia Ehr.	Europe	0	0	. 20	....
Tilia tomentosa Moench	Europe	0	0	. 10	. 19
Ulmus campestris L.	Europe & Orient	. 22	. 29	. 12	0
Ulmus effusa L.	Central & N. Europe	. 46	. 53	* 4	* 8
Ulmus montana With.	Europe & Asia	. 28	. 33	* 4	* 8
Vaccinium amoenum Ait.	N. America	. 21	. 43	. 4	. 10
Vergilia lutea Mx.	N. America	0	0	. 32	. 37
Viburnum Lantana L.	Europe & Orient	....	....	. 7	. 9
Vibusnum Opulus L.	Siberia, E. Asia & Amer.	. 26	. 34	. 7	. 14
Vitex Agnus-castus L.	Europe & Orient	. 14	. 33	. 9	. 15
Vitis riparia Michx.	N. America	. 34	. 39	. 11	0
Vitis vinifera L.	Orient	....	....	. 12	. 20
Weigela rosea Ldl.	Japan	. 8	. 15	. 9	. 14
Xanthoceras sorbifolia Bunge	China	0	0	0	0
Zanthorrhiza alpiifolia L'Herit	N. America	* 2	* 38	* 4	* 9
Zanthoxylum fraxineum Willd.	N. America	* 36	* 41	* 5	. 13

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A study of Table I shows the following results: Of the 234 species collected October 28 to November 4, 1905, a little more than half grew—that is, 125, while the remaining 109 made no growth.

The most of those that grew were European and Asiatic forms.

Of those that made no growth there were about equal numbers of American and European species, with also several from Asia, Japan, etc.

During the first nine days 42 species grew—that is, 18 per cent, those of European and Asiatic origin predominating, followed by American, Japanese and North African species in the order mentioned.

Of those collected in midwinter—January 8-10, 1906—the following results were taken from the table: There was a total of 283 species, of which 86 per cent—244, grew and 39 made no growth.

In the first nine days 142 species, that is, 50 per cent, grew, the highest number being of Asiatic origin, with European, American and Japanese forms following.

Since many kinds failed to grow in the two tests and a large number made but a slight growth, another set was collected on February 26, 1906. There was a total of 63 kinds in this list. In the same table—in the last two columns—is also the record of 10 more species brought in the 17th of March. This list was mainly those that made no growth in the immediately preceding test.

Table II contains the record of the collections of February 26 and March 17.

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TABLE II.

Twigs brought into the greenhouse without previous treatment.

. = Leafbud. * = Flowerbud. 0 = No growth.

SPECIES.	HABITAT.	Collected Feb. 26, 1906.		Collected March 17, 1906.	
		Growth began in	Buds fully open in	Growth began in	Buds fully open in
		Days	Days	Days	Days
Acer campestre L.	Europe & Orient	....	....	. 2	0
Acer campestre L., var. pulverulentum	Hort. form	....	....	. 8	0
Acer negundo L. var., crispum	N. Amer.	. 2	. 5	....	....
Acer Pseudo-Platanus L.	Europe	. 4	0	....	....
Acer spicatum Lam.	N. Amer.	. 3	. 7	....	....
Aesculus flava Ait.	N. Amer.	. 4	. 12	....	....
Aesculus humilis Lindl.	N. Amer.	. 3	0	....	....
Alnus viridis DC.	Europe, Asia & N. Amer.	. 15	* 22	. 3	. 24
Amorpha canescens Nutt.	N. Amer.	. 25	. 34	....	....
Amorpha fruticosa L.	N. Amer.	. 15	. 25	....	....
Azalea mollis Blume	China & Japan	. 3	. 11	....	....
Benzoin odoriferum Nas.	N. Amer.	. 3	. 13	....	....
Calycanthus floridus L.	N. & S. Carolina	. 3	. 8	....	....
Carya aquatica Nutt.	N. Amer.	0	0	. 3	0
Carya porcina Nutt.	N. Amer.	. 3	0	. 4	0
Castanea vesca Grtn.	S. Europe	. 3	. 12	....	....
Cercis canadensis L.	N. Amer.	. 3	. 8	....	....
Cercis Siliquastrum L.	Europe & Orient	. 5	. 8	....	....
Chionanthus fragrans Lindl.	N. America	. 2	0	....	....
Clethra acuminata Michx.	N. America	. 3	. 9	....	....
Colutea arborescens L.	Europe & Orient	0	0	....	....
Crataegus macracantha Lodd.	N. America	. 3	. 12	....	....
Cytisus laburnum L.	Europe	. 3	. 5	....	....
Diervilla canadensis Willd.	N. America	0	0	. 8	. 15
Diospyros virginiana L.	N. America	. 3	. 8	....	....
Elaeagnus argentea Pursh.	N. America	. 3	0	....	....
Erica carnea L.	S. Europe	0	0	....	....
Euonymus americana L.	N. America	. 7	. 19	....	....
Fagus sylvatica L.	Europe & W. Asia	. 27	0	. 20	. 29
Fraxinus americana L.	N. America	. 8	. 63	. 2	. 7
Fraxinus excelsior L.	Europe & W. Asia	. 11	0	. 8	. 22
Fraxinus oregona Nutt.	N. America	. 6	. 11	....	....
Fraxinus Ornus L.	S. Europe	0	0	0	0
Hamamelis virginiana L.	N. America	. 5	. 8	....	....
Hydrangea radiata Walt.	N. America	. 2	. 6	....	....
Jasminum fruticans L.	S. Europe, N. Afr. & Orient.	. 1	. 5	....	....
Juglans regia L.	Europe & Asia	. 6	. 12	....	....
Kalmia angustifolia L.	N. America	* 8	0	....	....

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TABLE II. (*Continued.*)

Twigs brought into the greenhouse without previous treatment.

. = Leafbud. * = Flowerbud. 0 = No growth.

SPECIES.	HABITAT.	Collected Feb. 26, 1906.		Collected March 17, 1906.	
		Growth began in	Buds fully open in	Growth began in	Buds fully open in
		Days	Days	Days	Days
Koelreuteria paniculata L.	China	. 8	. 8	....	....
Ledum latifolium Jacq.	N. America	. 2	. 12	....	....
Liriodendron tulipifera L.	N. America	. 6	. 12	....	....
Myrica Gale L.	Europe & N. America	* 8	* 6	....	....
Ostrya vulgaris Willd.	S. Europe & Orient	. 8	. 8	....	....
Ptelea trifoliata L.	N. America	. 7	. 84	....	....
Pyrus Aria, var., edulis Ehr.	Europe & Asia	. 8	. 11	....	....
Quercus alba L.	N. America	. 6	0	....	....
Quercus ambigua W.	N. America	. 8	0	....	....
Quercus Cerris L.	S. Europe & Asia	. 6	. 13	....	....
Quercus coccinea Wang.	N. America	. 7	0	....	....
Quercus Daborochensis C. Koch.	Europe	. 7	0	....	....
Quercus imbricaria Michx.	N. America	. 6	. 11	....	....
Quercus macranthera F. & M.	Caucasus	. 12	. 16	....	....
Quercus macrocarpa Michx.	N. America	. 11	. 20	....	....
Quercus olivaeformis Michx.	N. America	. 12	. 20	....	....
Rhododendron ponticum, var. Cunninghami L...	S. Europe & Asia Minor..	. 11	0	....	....
Robinia Pseud-acacia L.	N. America	. 6	. 13	....	....
Robinia Pseud-acacia, var. semperflorens L. ..	N. America	. 8	. 9	....	....
Salisburia adiantifolia Sm.	China & Japan	. 8	. 12	....	....
Sorbus Japonica L.	China & Japan	. 6	. 11	....	....
Tilia alba Ait.	N. America	. 8	. 12	....	....
Tilia argentea DC.	Europe	. 2	. 8	....	....
Tilia euchlora C. Koch.	Orient	. 8	. 8	....	....
Tilia handwort	Siberia	. 8	. 16	....	....
Vitis riparia Michx.	N. America	. 8	. 19	....	....
Xanthoceras sorbifolia Bunge	China	. 8	0	....	....

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Of the list of 63 kinds collected February 26 (Table II), all grew but 5. At this time growth occurred in most instances very quickly, 49 having begun to grow during the first nine days, and 7 more within the second nine days, thus leaving but two more to occupy a longer time for growth. The American species, on the average, were later in beginning to grow than either the Asiatic or European.

The final test consisting of ten species, collected March 17, repsented mainly those that had not grown before. Nine of the number grew.

TREATMENTS FOR FORCING GROWTH.

2. *The Use of Ether Only* (Tables III, IV and V).

Time of treatment, from November 17 to 24, 1905.

Treated with ether only, as follows:

Etherized 48 hours
" 48+48 hours
" 72 hours
Control.

It should be explained that "48+48" means that the plants were exposed to the ether fumes for 48 hours, removed to the greenhouse and kept standing in water for 48 hours and then returned to the ether vapor for another 48 hours.

While being etherized the twigs were not kept in water, merely being enclosed in the large jars just as they came from the garden. It probably would have been better to have had the plants in water during the longer periods of etherization, but as ether vapor is readily absorbed by water, it was not practicable to do so.

During treatment the jars containing the twigs were on the floor between two windows and in this place the temperature at night sometimes fell as low as 17° or even 16°. The usual temperature, however, was between 18° and 20° C.

This was the first attempt to force growth. The list treated numbered 56 species. Here as before, figures given in the table refer to number of days required for growth to begin and for buds to open. The same characters are also used to indicate whether the growth was for a leaf bud or a flower bud. The details follow in Table III:

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Treated with ether only from November 17-24, 1905

. = Leafbud. * = Flowerbud. 0 = No growth.

SPECIES.	HABITAT.	Control.		Etherised 48 hours.		Etherised 48 + 48 hours.		Etherised 72 hours.	
		Growth began in	Buds fully open in	Growth began in	Buds fully open in	Growth began in	Buds fully open in	Growth began in	Buds fully open in
		Days	Days	Days	Days	Days	Days	Days	Days
Aesculus Hippocastanum L.	Orient	0	0	* 23	0	. 21	* 39	* 22	* 32
Amelanchier ovalis Lindl.	N. America	* 13	0	. 4	0	* 1	0	* 3	0
Amelanchier vulgaris Moench.	S. Europe	0	0	. 16	0	* 6	0	0	0
Broussonetia papyrifera Vent.	China & Japan	* 12	* 17	. 4	0	. 3	0	. 6	0
Calophaca wolgarica Fisch.	S. Russia	. 8	. 13	. 4	. 10	* 6	. 14	. 4	. 9
Caragana arborescens Lam.	Siberia	. 8	0	. 4	. 10	. 1	0	. 4	* 10
Castanea pumila Michx.	N. America	0	0	0	0	0	0	. 21	. 27
Castanea vesca Grtn.	S. Europe	. 25	. 34	0	0	0	0	. 15	. 25
Cercis canadensis L.	N. America	. 17	0	0	0	. 7	0	0	0
Colutea arborescens L.	Europe & Orient	0	0	. 4	0	0	0	0	0
Cornus Mas. L.	Cent. & S. Europe	* 11	* 14	. 10	0	. 7	. 13	. 10	. 15
Crataegus macracantha Lodd.	N. America	0	0	* 40	0	0	0	0	0
Crataegus Oxyacantha L.	Europe	0	0	. 2	. 10	* 3	0	. 10	0
Crataegus spinosissima Lodd.	Siberia	0	0	. 1	. 11	0	0	. 6	. 15
Cydonia japonica Pers.	Japan	. 5	. 8	0	0	. 7	. 12	0	0
Cydonia vulgaris L.	S. Europe	. 10	. 13	. 1	. 15	0	0	. 10	. 13
Cytisus weldeni Vis.	S. Hungary	* 7	0	0	0	0	0	* 4	0
Ginkgo biloba L.	Japan	0	0	* 26	* 43	* 34	* 45	0	0
Halimodendron argentium Fisch.	Siberia & Tar.	. 8	. 21	. 5	. 12	. 3	. 8	. 4	0
Magnolia Soulangeana Soul.	Japan	. 17	. 34	. 5	0	* 5	* 12	. 11	. 15
Platanus orientalis L.	Europe & Orient	. 29	0	. 21	* 27	* 18	* 26	. 4	. 17

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TABLE III. (*Continued.*)

Treated with ether only from November 17-24, 1905.

. = Leafbud. * = Flowerbud. 0 = No growth.

SPECIES.	HABITAT.	Control.		Etherized 48 hours.		Etherized 48 + 48 hours.		Etherized 72 hours.	
		Growth began in	Buds fully open in	Growth began in	Buds fully open in	Growth began in	Buds fully open in	Growth began in	Buds fully open in
		Days	Days	Days	Days	Days	Days	Days	Days
Prunus Armeniaca L.	Caucasus	* 12	0	0	0	* 11	0	. 10	0
Prunus padus L.	Europe & Asia	0	0	* 2	0	* 1	0	* 4	0
Prunus serotina Ehr.	N. America	* 10	0	* 5	* 14	* 1	. 13	* 4	0
Prunus virginiana L.	N. America	. 23	. 29	. 4	. 10	. 4	. 10	* 5	0
Pyrus americana DC.	N. America	0	0	0	0	0	0	. 14	. 18
Pyrus Aria, var. edulis Ehr.	Europe & Asia	. 54	. 59	* 39	0	0	0	0	0
Pyrus aucuparia Ehr.	Europe & Asia	. 23	. 30	. 5	. 15	. 3	. 8	. 4	. 6
Pyrus malus L.	Europe & Asia	* 13	. 30	* 10	0	. 7	. 15	* 9	0
Pyrus microcarpa DC.	N. America	. 39	. 45	0	0	. 21	. 27	. 11	. 15
Pyrus prunifolia Willd.	Siberia	. 14	. 21	. 10	. 18	. 3	. 11	. 4	. 15
Quercus ambigua W.	N. America	0	0	0	0	. 11	. 25	0	0
Quercus Dahorochensis C. Koch	Europe	. 25	. 33	0	0	0	0	. 12	. 19
Quercus macranthera F. & M.	Caucasus	0	0	. 22	0	. 11	. 21	0	0
Quercus macrocarpa Michx.	N. America	. 45	0	. 29	0	0	0	0	0
Quercus ramosa Booth	N. America	. 43	0	. 23	. 31	0	0	0	0
Rhododendron ponticum, var. Cunninghami, L.	S. Europe & Asia Minor	0	0	. 16	. 19	0	0	0	0
Robinia Pseud-acacia, var. amorphaefolia L.	N. America	. 16	. 20	. 10	0	. 6	0	. 8	. 14
Sorbus graeca Lodd. & Steud.	Europe & Asia	0	0	. 28	. 55	0	0	. 14	. 29
Spiraea Lindleyana Wall.	Asia	. 3	. 18	. 3	. 14	. 3	. 11	. 3	. 13
Spirea sorbifolia L.	Asia	. 6	. 7	. 1	. 3	. 1	. 1	. 2	. 3

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Fifty-six species were treated with ether in Table III, of which 42 grew and 14 did not. Only the 42 that grew were included in the table. The fourteen that did not grow were the following:

Amelanchier canadensis Medic., North Amer.
Amorpha canescens Nutt., North Amer.
Amorpha fruticosa L., North Amer.
Cercis Siliquastrum L., Europe and Orient.
Cotoneaster vulgaris Lal., Europe and Orient.
Erica carnea L., South Europe.
Fagus sylvatica L., Europe and North Asia.
Gleditschia triacanthos L., North Amer.
Ostrya vulgaris Willd., South Europe and Orient.
Prunus Persica Stokes, Asia.
Quercus alba L., North Amer.
Quercus olivaeformis Michx., North Amer.
Quercus pubescens W., Europe and Asia.
Vergilia lutea Mx., North Amer.

Of the 42 species that grew all made some growth from one or more of the treatments, while only a little more than half (64.2%) of the same species grew without treatment.

A clearer idea of the outcome of this experiment will be gained from the following brief summary which shows in condensed form the results of all of the treatments on the entire 56 species, with the control included for comparison:

TABLE IV.

Summary of Table III. Fifty-six species; treated, November 17-24, 1905.

TREATMENT.	Average length of time required to begin growth.	Average length of time required for buds to open fully.	Percentage that grew.	Percentage that opened their buds fully.
	Days.	Days.		
Control	18.5	22.5	48.2	32.1
Etherized 48 hrs.	12.0	18.6	56.0	30.8
Etherized 48 + 48 hrs.	8.2	14.2	50.0	32.1
Etherized 72 hours.....	8.8	16.3	50.0	33.9

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The marked effects of the ether are seen in the larger percentage that grew when treated, and especially in the shorter time required for growth to begin. A gain in time of growth of more than ten days followed the 48+48 hours and the 72 hours treatments, while there was but 6.5 days gain from the 48 hours treatment. Etherizing for 48+48 hours gave the best results in this test.

While the summary just given shows the average time required for growth to begin under each treatment, it does not show the true effects of treatments in their proper relations. The chief defect lies in the fact that, of necessity, direct comparison was made between easily reacting species and those that grew with great difficulty even under severe treatment. For example, in the control *Cydonia japonica* grew first in five days, while *Pyrus aria,* variety *edulis,* also untreated, did not begin to grow until fifty-four days had elapsed. And, when treated with ether for forty-eight hours, *Pyrus aria,* var. *edulis,* required thirty-nine days to begin growth, while the *Cydonia vulgaris,* under the same treatment, grew in one day. Manifestly, then, it is unfair and does not show the exact or anywhere near the exact state of affairs in either case to take an average between the five days and fifty-four days, in the one instance, or the one day and the thirty-nine days in the other. This had to be done, however, in order to reduce the results to a form brief enough to admit of easy comparisons. The summary referred to serves its purpose but realizing its defects another review was prepared as follows:

TABLE V.

Second Summary of Table III, showing the extent of growth at the end of 1 wk., 2 wks., 3 wks., and, after 3 weeks.

TREATMENT.	Percentage that began growth in				Percentage that opened their b fully in			
	1 week.	2 weeks.	3 weeks.	More than 3 weeks.	1 week.	2 weeks.	3 weeks.	More than
Control	11.1	44.4	11.1	33.6	5.55	22.2	27.75	4
Etherized 48 hrs.	50.0	15.62	9.37	25.0	5.88	47.05	23.52	2
Etherized 48 + 48 hours	71.4	10.71	10.71	7.14	5.55	55.5	11.10	2
Etherized 72 hrs.	48.13	41.26	6.88	3.44	10.53	26.32	42.10	2

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In this second summary just given the number of days elapsing before growth began was divided into groups or periods. The shortest period was one week, that is, all that began to grow within seven days after treatment were grouped together and in like manner all that grew inside of two weeks, three weeks, and those requiring more than three weeks respectively were grouped together.

This method shows some results not brought out before. For instance, in the first summary the effects from etherizing 48+48 hours and of 72 hours appear to be practically the same (8.2 and 8.3 days), but in the second or more detailed review it is seen that of those treated for 48+48 hours 71.4 per cent grew inside of seven days, while of those etherized 72 hours only 48.13 per cent grew in the same length of time. This means that the ether acting continuously through a long period of time, although seemingly as effective as the 48+48 hours treatment, did not cause a quick growth in as large a number of plants as the latter. It is furthermore plainly shown that 48+48 hours of ether caused practically all the number in the list to begin growth during the first week after treatment while those etherized 72 hours required two weeks for accomplishing the same results. On the other hand, the untreated plants and those receiving 48 hours of ether were slow in starting into growth, one-third of the former and one-fourth of the latter beginning to grow after three weeks from date of treatment.

Regarding the effects of the treatments on the time required for the buds to fully open, it is noticed that at the end of three weeks almost half of the controls had not opened, while at the same time a much smaller number of the treated ones were not yet out. It is further interesting to follow all the way through the effects of etherizing for 48+48 hours. Although nearly three-fourths of the species began to grow inside of one week, one-fourth of the buds had not opened fully after three weeks. This seems to indicate that with many species the treatment may cause such a stimulus to the buds that they are quickly started into action but that the growth does not continue until the buds (flower or leaf) are fully open. Observations during experiments also confirm this.

3. *Experiments with Freezing, Etherizing and Use of Dark-Chamber* (Tables VI, VII and VIII.)

Time of treatement from November 21 to December 1, 1905. The 35 species were given the following treatments:

Frozen for 7 days;
Frozen for 21 days;
Frozen for 7 days, then etherized for 24 hours;
Frozen for 21 days, then etherized for 48 hours;

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In the moist, dark-chamber for 8 days;
In the moist, dark-chamber for 17 days;
Control.

This was the second attempt to force growth. Ether alone having been found to be effective in awakening many plants from their dormant state, two new methods of treatment—freezing and confining plants in a moist, dark-chamber, were introduced. It was desired also to find if the ether would exert any more influence when used immediately after the plants were severely frozen than would the freezing alone. The dark-chamber supplied two conditions not present in the greenhouse, viz., uniform temperature and high, constant humidity.

In Table VI, which follows, are to be seen in detail the results of all the treatments:

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Treated between November 21 and December 1, 1905.

. = Leafbud. * = Flowerbud. 0 = No growth.

SPECIES.	HABITAT.	Control.		Frosen 7 days.		Frosen 21 days.		Frosen 7 days; Etherised 24 hours.		Frosen 7 days: Etherised. 48 hours.		Dark Chamber 8 days.		Dark Chamber 17 days.	
		Growth began in	Buds fully open in	Growth began in	Buds fully open in	Growth began in	Buds fully open in	Growth began in	Buds fully open in	Growth began in	Buds fully open in	Growth began in	Buds fully open in	Growth began in	Buds fully open in
		Days	Days	Days	Days	Days	Days	Days	Days	Days	Days	Days	Days	Days	Days
Acer macrophyllum Pursh.	N. America	0	0	0	0	0	0	0	0	0	0	. 41	. 45	0	0
Acer platanoides L.	Europe	0	0	0	0	. 20	. 35	. 41	. 53	0	0	. 48	. 55	. 42	0
Aesculus Hippocastanum L.	Orient	* 21	* 33	. 31	. 44	. 22	. 35	. 22	. 32	. 19	. 26	. 15	. 29	. 15	* 30
Alnus barbata C. A. Mey	Caucasus	. 22	. 27	0	0	0	0	0	0	0	0	0	0	0	0
Amorpha canescens Nutt.	N. America	* 21	* 29	0	0	* 28	. 36	0	0	0	0	* 24	. 45	. 23	. 29
Amygdalus communis L.	Orient	. 9	. 18	. 5	* 19	. 7	* 15	. 6	. 12	. 3	. 9	0	0	0	0
Azalea pontica L.	Asia Minor	* 26	* 37	. 35	. 58	0	0	0	0	0	0	. 9	. 13	. 39	* 44
Betula alba L.	Europe & Asia	. 17	. 23	. 11	. 18	. 13	* 13	. 6	. 18	. 4	. 9	. 11	. 15	. 11	. 15
Caragana arborescens Lam.	Siberia	. 9	0	. 4	. 11	0	0	. 3	0	. 3	0	0	0	0	0
Carpinus americana Michx.	N. America	0	0	. 35	. 45	0	0	. 31	. 41	. 19	. 27	0	0	0	0
Cornus Mas L.	Europe	* 9	* 11	* 4	* 7	* 1	* 7	* 10	0	. 11	. 17	* 11	* 15	* 11	* 15
Corylus Colurna L.	Asia Minor	0	* 15	0	0	0	0	0	0	0	0	0	* 19	0	* 19
Crataegus macracantha Lodd.	N. America	. 53	0	0	0	0	0	. 33	. 43	0	0	0	0	. 52	0
Gleditschia triacanthos L.	N. America	. 11	. 16	0	0	0	0	0	0	0	0	. 12	0	0	0
Populus candicans Ait.	Asia & N. Amer.	. 19	. 25	. 13	. 18	. 15	. 19	. 13	. 18	* 4	* 9	. 24	. 27	. 22	. 25
Populus monilifera Rozier	N. America	. 33	. 42	. 26	. 33	. 21	. 33	. 26	. 32	. 23	. 29	. 27	. 33	. 21	. 25
Prunus cerasus L.	Asia Minor	* 11	* 16	* 5	. 19	. 5	. 14	. 3	. 18	* 4	. 17	* 10	. 19	* 9	. 18
Prunus domestica L.	Caucasus	0	0	0	0	. 16	. 23	0	0	0	0	0	0	0	0
Prunus virginiana L.	N. America	. 25	. 31	* 17	0	* 21	0	* 6	. 22	0	0	* 29	0	* 39	0
Pyrus cerasifera Tausch.	Asia Minor	. 9	. 15	. 13	. 20	. 24	. 35	. 13	. 18	. 5	. 16	* 11	0	. 11	. 31

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TABLE VI. (*Continued.*)

Treated between November 21 and December 1, 1905.

. = Leafbud. * = Flowerbud. 0 = No growth.

SPECIES.	HABITAT.	Control.		Frosen 7 days.		Frosen 21 days.		Frosen 7 days; Etherized 24 hours.		Frosen 7 days; Etherized. 48 hours.		Dark Chamber 8 days.		Dark Chamber 17 days.	
		Growth began in	Buds fully open in	Growth began in	Buds fully open in	Growth began in	Buds fully open in	Growth began in	Buds fully open in	Growth began in	Buds fully open in	Growth began in	Buds fully open in	Growth began in	Buds fully open in
		Days	Days	Days	Days	Days	Days	Days	Days	Days	Days	Days	Days	Days	Days
Pyrus communis L.	Europe & Asia	* 9	. 15	* 6	. 19	. 5	. 13	* 7	. 14	* 3	. 19	. 6	. 14	. 6	. 14
Pyrus malus L.	Europe & Asia	. 11	. 16	. 13	. 18	. 23	0	. 9	. 18	0	0	. 11	. 18	. 11	. 17
Pyrus microcarpa DC.	N. America	. 21	. 28	. 15	. 23	. 20	. 27	. 14	. 22	. 10	. 18	* 15	. 26	. 16	. 28
Pyrus prunifolia Willd.	Siberia	. 11	. 16	. 12	. 22	. 5	. 13	. 6	. 12	. 8	. 16	. 12	. 17	. 12	. 19
Quercus Dahorochensis C. Koch.	Europe	* 17	0	0	0	0	0	0	0	0	0	0	0	0	0
Robinia Pseud-acacia, var. amorphaefolia L.	N. America	0	0	0	0	0	0	0	0	0	0	0	0	. 19	. 25
Salix alba vitellina pendula L.	Asia & Africa	. 7	. 11	. 6	. 9	. 6	. 10	. 3	. 8	. 9	. 13	. 3	. 9	. 3	. 9
Sophora Japonica L.	Japan & China	0	0	0	0	0	0	. 13	. 17	0	0	. 14	. 18	0	0
Sorbus graeca Lodd. & Steud.	Europe & Asia	0	0	0	0	0	0	. 29	. 37	. 27	. 36	0	0	. 46	. 51
Syringa vulgaris L.	Orient	. 17	. 29	. 6	. 12	. 10	. 14	. 5	. 9	0	0	. 13	. 23	0	0
Vergilia lutea Michx.	N. America	0	0	0	0	0	0	. 38	. 42	. 14	. 20	0	0	0	0
Viburnum Lantana L.	Europe & Orient	. 26	. 29	. 7	. 12	0	0	. 22	0	0	0	. 28	. 31	. 27	. 29

In Table VI, only three species out of the 35 made no growth whatever:

Acer dasycarpum Ehr., North Amer.,
Amorpha fruticosa L., North Amer., and
Fagus sylvatica L., Europe and W. Asia.

Of the 32 species given in Table VI, 93.7 per cent grew from one or more treatments, while only 65.7 per cent grew without treatment. A fairer method of considering the results would be to compare the control with each of the treatments separately. This method will give the relative merits of each treatment while comparing the control with all of the treatments merely shows in general that the sum of many conditions gave better results than one condition.

In the last table only the 32 species that grew were given but in the following summary of Table VI, all the 35 species treated are considered, including the control:

TABLE VII.

Summary of Table VI. Thirty-five species; treated between November 21 and December 1, 1905.

. = Leafbud. * = Flowerbud. 0 = No growth.

TREATMENT.	Average length of time required for beginning growth.	Average length of time required for buds to open fully.	Percentage that grew.	Percentage that opened their buds fully.
	days.	days.		
Control	18.0	21.7	65.7	60.0
Frozen 7 days	14.4	22.6	54.2	51.4
Frozen 21 days	14.2	21.8	51.4	45.7
Frozen 7 days; etherised 24 hours	15.6	24.3	65.7	57.1
Frozen 7 days; etherised 48 hours	10.3	18.7	45.7	42.8
In moist dark chamber 8 days	17.7	24.7	60.0	54.2
In moist dark chamber 17 days	21.7	24.6	57.1	51.4

The review shows some very interesting results. No. 5 made the quickest growth, average 10.3 days, a gain over the control of 7.7 days.

Freezing for one week and for three weeks (No. 2 and No. 3) caused about the same results in length of time required for growth and the percentage that grew.

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Comparing No. 2 and No. 4 it is seen that the addition of the ether did not hurry the growth but the increase in the number of those that grew was more than 11 per cent.

Comparing 3 and 5, similar treatments except for the ether, the anesthetic seems to have exerted an extraordinary effect in two ways: first, the time was much shortened; and, second, a smaller percentage grew.

In every treatment but one—No. 4—a smaller percentage grew than the untreated. On the other hand, every treatment but one—No. 7—caused a quicker growth than the control.

It will further be noticed that the treatment giving the shortest time for growth (No. 5, gain 7.7 days) shows the *smallest* number that grew, loss 20 per cent! This is true in less degree with nearly all of the treatments.

Evidently freezing, followed by strong etherization, exerts a profound influence on dormant plants, causing them either to grow and grow quickly or to be killed or severely injured. There was little evidence of any of the plants remaining indifferent to the treatments.

More than half of the species grew without treatment—65.7 per cent. In no case did a treatment cause a larger percentage of growth than the untreated and only in one instance—No. 4—was it equaled.

Those species that grew without treatment and also many that forced easily, were often injured by the severe treatments. In other words, they responded readily to the treatments, but when prolonged, as in No. 5, many were killed and thus the percentage that grew would be very small. This is the explanation for the small percentage, in general, of the treated ones that grew. No. 4, however, seems to have been an exception.

Below will be seen a second summary of the last experiment in which the time required for growth to begin is again divided into weekly periods up to three weeks, when all that grew after that are grouped under the heading of "more than 3 weeks."

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TABLE VIII.

Second Summary of Table VI, showing the extent of growth at the end of 1 wk., 2 wks., 3 wks., and, after 3 wks.

TREATMENT.	Percentage that began growth in				Percentage that opened their buds fully in			
	1 week.	2 weeks.	3 weeks.	More than 3 weeks.	1 week.	2 weeks.	3 weeks.	More than 3 weeks.
1 Control	4.35	39.12	30.43	26.08	0	15.79	36.85	57.89
2 Frozen 7 days	42.10	26.31	10.53	21.05	5.55	22.20	38.85	33.30
3 Frozen 21 days	33.30	11.10	33.30	22.20	5.88	35.29	11.76	47.05
4 Frozen 7 days; etherised 24 hours	39.12	26.08	0	34.78	0	25.0	30.0	45.0
5 Frozen 7 days; etherised 48 hours	43.75	31.25	12.50	13.50	6.67	26.66	46.67	26.66
6 Dark Chamber 8 days	9.52	47.61	9.52	33.33	0	15.79	36.84	47.37
7 Dark Chamber 17 days	10.0	30.0	20.0	40.0	0	11.10	33.30	55.50

The most noticeable thing about this review is the large percentage that made a quick growth from treatment No. 5 (frozen 7 days then etherized 48 hours). This was the treatment that caused growth in the shortest average length of time, and it is now found—as before—that the first week saw the beginning of growth in a great majority of the species. Unlike the previous experiment, however, the growth in this case was not of a temporary nature, but continued until the buds were fully open.

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A queer feature brought out by the table was the behavior of those frozen seven days. Just why a large percentage of the species should have made a quick growth there is not clear when the average number of days required for growth, as shown in Table VII, was 14.4 days or 4.1 days more than the No. 5 treatment.

Taking up the first column in the summary, the percentage of species that grew inside of one week, and comparing the control with each of the others the full effects of the different treatments are seen in a striking manner.

4. *Experiment in Etherizing, Drying, Freezing and Dark-Chamber, Singly and in Combination* (Tables IX, X and XI).

Date of treatment December 8 to 23, 1905; number of species 70. The treatments were as follows:

Etherized 48 hours;
Etherized 48+48 hours;
Dried 1 day;
Dried 2 days;
Dried 3 days;
Dried 4 days;
Dried 5 days;
Frozen 8 days;
Frozen 14 days;
Confined in dark-chamber 8 days;
Confined in dark-chamber 14 days;
Frozen 8 days, then etherized 48 hours;
Frozen 8 days, then etherized 72 hours;
Frozen 8 days, then in dark-chamber 5 days;
In dark-chamber 8 days, then etherized 48 hours;
In dark-chamber 8 days, then etherized 72 hours;
Control.

This was the last of the three main sets of treatments, and was by far the largest of them all, both in point of number of species used and the number of treatments employed. Ether and freezing, singly and in combination, having proved beneficial in forcing growth and an independent test in drying (not shown here—see page 78) having shown some interesting results, it was desired to compare the effects of these three treatments in particular. At the same time the dark-chamber was tried again by itself and also followed by etherization. The full results are given in detail in Table IX which follows:

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TABLE IX.

Treated from December 8 to 23, 1905.

. = Leafbud. * = Flowerbud. 0 = No growth.

TREATMENT.		Acer campestre L. Europe & Orient.	Acer campestre L., var. pulverulentum. Hort. form.
		Days.	Days.
Control	Began growth in	. 20	0
	Buds fully open in	. 25	0
Etherised 48 hours	Began growth in	0	. 9
	Buds fully open in	. 0	. 32
Etherised 48 + 48 hours	Began growth in	0	. 12
	Buds fully open in	0	0
Dried 1 day	Loss of weight	4.60%	9.52%
	Began growth in	0	0
	Buds fully open in	0	0
Dried 2 days	Loss of weight	6.85%	8.97%
	Began growth in	. 17	0
	Buds fully open in	0	0
Dried 3 days	Loss of weight	9.56%	11.26%
	Began growth in	* 14	* 17
	Buds fully open in	0	0
Dried 4 days	Loss of weight	12.15%	16.66%
	Began growth in	0	0
	Buds fully open in	0	0
Dried 5 days	Loss of weight	14.92%	18.60%
	Began growth in	* 9	0
	Buds fully open in	. 21	0
Frozen 8 days	Began growth in	. 26	. 14
	Buds fully open in	0	0
Frozen 14 days	Began growth in	0	0
	Buds fully open in	0	0
In dark chamber 8 days	Began growth in	. 20	. 30
	Buds fully open in	. 27	. 38
In dark chamber 14 days	Began growth in	. 23	. 24
	Buds fully open in	0	. 37
Frozen 8 days; etherised 48 hours	Began growth in	. 30	0
	Buds fully open in	0	0
Frozen 8 days; etherised 72 hours	Began growth in	0	. 10
	Buds fully open in	0	. 16
Frozen 8 days; in dark chamber 5 days	Began growth in	. 26	0
	Buds fully open in	0	0
Dark chamber 8 days; etherised 48 hours	Began growth in	. 22	. 17
	Buds fully open in	0	. 34
Dark chamber 8 days; etherised 72 hours	Began growth in	0	. 26
	Buds fully open in	0	. 32

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TABLE IX. (*Continued.*)

Treated from December 8 to 23, 1905.

. = Leafbud. * = Flowerbud. 0 = No growth.

TREATMENT.		Acer dasycarpum Ehr. N. America.	Acer monspessulanum L. S. Europe.
		Days.	Days.
Control	Began growth in	. 25	0
	Buds fully open in	0	0
Etherized 48 hours	Began growth in	0	0
	Buds fully open in	0	0
Etherized 48 + 48 hours	Began growth in	0	0
	Buds fully open in	0	0
Dried 1 day	Loss of weight	4.60%	4.95%
	Began growth in	* 15	0
	Buds fully open in	* 22	0
Dried 2 days	Loss of weight	7.14%	8.91%
	Began growth in	0	0
	Buds fully open in	0	0
Dried 3 days	Loss of weight	9.84%	12.90%
	Began growth in	0	0
	Buds fully open in	0	0
Dried 4 days	Loss of weight	21.31%	15.17%
	Began growth in	0	0
	Buds fully open in	0	0
Dried 5 days	Loss of weight	23.26%	21.90%
	Began growth in	* 12	0
	Buds fully open in	* 18	0
Frozen 8 days	Began growth in	0	0
	Buds fully open in	0	0
Frozen 14 days	Began growth in	0	0
	Buds fully open in	0	0
In dark chamber 8 days	Began growth in	0	0
	Buds fully open in	0	0
In dark chamber 14 days	Began growth in	0	. 18
	Buds fully open in	0	. 25
Frozen 8 days; etherized 48 hours	Began growth in	0	0
	Buds fully open in	0	0
Frozen 8 days; etherized 72 hours	Began growth in	0	0
	Buds fully open in	0	0
Frozen 8 days; in dark chamber 5 days	Began growth in	0	0
	Buds fully open in	0	0
Dark chamber 8 days; etherized 48 hours	Began growth in	0	. 23
	Buds fully open in	0	0
Dark chamber 8 days; etherized 72 hours	Began growth in	. 39	. 20
	Buds fully open in	. 47	. 30

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TABLE IX. (*Continued.*)

Treated from December 8 to 23, 1905.

. = Leafbud. * = Flowerbud. 0 = No growth.

Acer negundo L., var. californicum. California.	Acer negundo L., var. versicolor. N. America.	Acer negundo L. var. violaceum. N. America.	Acer Pseudo-Platanus L. Europe.	Acer spicatum Lam. N. America.	Acer Tataricum L. S. E. Europe.
Days.	Days.	Days.	Days.	Days.	Days.
0	. 33	. 39	0	0	. 27
0	. 42	. 45	0	0	. 36
. 17	0	. 9	. 18	. 10	. 8
. 24	0	. 26	0	. 22	0
. 7	0	. 17	* 11	. 7	0
. 30	0	0	* 18	. 22	0
5.60%	3.77%	4.13%	6.90%	7.31%	7.72%
. 48	. 31	. 34	* 20	0	* 12
0	. 43	0	* 29	0	* 21
7.18%	9.34%	6.83%	8.45%	9.19%	11.76%
. 48	.29	. 37	0	0	* 11
0	. 42	0	0	0	* 20
12.31%	14.96%	6.07%	3.41%	12.64%	16.82%
. 15	.21	. 32	* 16	0	0
. 22	. 32	0	* 24	0	0
12.73%	16.66%	14.45%	11.04%	14.15%	17.93%
. 47	. 16	. 23	. 14	0	0
. 52	. 28	0	. 39	0	0
10.52%	19.65%	17.64%	15.66%	12.50%	19.0%
. 24	. 12	. 17	0	. 18	0
0	. 20	. 25	0	. 26	0
0	0	0	0	0	0
0	0	0	0	0	0
0	0	0	0	0	0
0	0	0	0	0	0
. 35	0	. 26	0	0	* 21
0	0	. 36	0	0	0
. 30	. 32	. 23	0	. 24	* 22
. 37	0	. 37	0	0	0
. 13	. 13	. 13	0	0	0
0	0	0	0	0	0
. 11	0	. 10	0	. 10	0
. 24	0	0	0	0	0
0	0	. 16	0	0	0
0	0	0	0	0	0
. 20	. 32	. 20	. 21	. 19	. 8
0	0	. 34	0	. 25	0
. 29	0	. 21	0	. 18	0
. 34	0	0	0	. 24	0

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TABLE IX. (*Continued.*)

Treated from December 8 to 23, 1905.

. = Leafbud. * = Flowerbud. 0 = No growth.

TREATMENT.		Betula nigra L. N. America.	Castanea pumila Michx. N. America.
		Days.	Days.
Control	Began growth in	. 26	0
	Buds fully open in	. 32	0
Etherised 48 hours	Began growth in	* 7	0
	Buds fully open in	* 12	0
Etherised 48 + 48 hours	Began growth in	. 4	0
	Buds fully open in	. 11	0
Dried 1 day	Loss of weight	7.95%	14.28%
	Began growth in	. 26	0
	Buds fully open in	. 37	0
Dried 2 days	Loss of weight	12.79%	10.87%
	Began growth in	. 26	0
	Buds fully open in	. 37	0
Dried 3 days	Loss of weight	14.11%	15.08%
	Began growth in	. 24	0
	Buds fully open in	. 29	0
Dried 4 days	Loss of weight	18.93%	12.72%
	Began growth in	. 24	0
	Buds fully open in	. 32	0
Dried 5 days	Loss of weight	20.77%	19.58%
	Began growth in	. 21	0
	Buds fully open in	. 27	0
Frozen 8 days	Began growth in	. 29	0
	Buds fully open in	* 34	0
Frozen 14 days	Began growth in	0	0
	Buds fully open in	0	0
In dark chamber 8 days	Began growth in	* 22	0
	Buds fully open in	* 26	0
In dark chamber 14 days	Began growth in	0	0
	Buds fully open in	0	0
Frozen 8 days; etherised 48 hours	Began growth in	0	0
	Buds fully open in	0	0
Frozen 8 days; etherised 72 hours	Began growth in	0	0
	Buds fully open in	0	0
Frozen 8 days; in dark chamber 5 days	Began growth in	. 30	0
	Buds fully open in	. 36	0
Dark chamber 8 days; etherised 48 hours	Began growth in	. 13	. 25
	Buds fully open in	. 21	0
Dark chamber 8 days; etherised 72 hours	Began growth in	. 12	0
	Buds fully open in	. 20	0

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TABLE IX. (*Continued.*)

Treated from December 8 to 23, 1905.

.=*Leafbud.* *=Flowerbud. 0=No growth.

Juglans nigra L. N. America.	Liquidambar styraciflua L. N. America.	Morus germanica L. Europe.	Ostrya vulgaris Willd. S. Europe & Orient.	Platanus orientalis L. Europe & Orient.	Populus balsamifera Lyall. California.
Days.	Days.	Days.	Days.	Days.	Days.
. 12	. 35	. 21	0	. 22	. 16
. 31	. 45	. 28	0	. 32	. 23
. 28	0	. 6	0	. 16	. 7
. 34	0	. 12	0	. 23	. 13
. 22	. 27	. 16	. 14	. 18	. 10
. 23	. 35	0	0	. 29	. 16
3.36%	12.16%	6.83%	8.54%	6.52%	4.54%
. 31	0	. 14	0	* 17	. 12
0	0	. 23	0	* 26	. 19
5.49%	16.21%	11.46%	9.30%	11.22%	6.76%
. 29	0	. 10	. 39	* 14	. 7
. 30	0	. 18	0	0	. 19
8.53%	20.14%	12.26%	10.93%	13.65%	10.24%
. 25	0	. 10	0	0	. 11
0	0	. 17	0	0	. 18
10.23%	25.15%	15.44%	12.03%	13.98%	8.97%
0	0	0	0	* 23	. 10
0	0	0	0	. 25	. 19
9.05%	30.11%	14.77%	16.09%	20.13%	16.71%
0	0	. 9	. 14	. 0	. 10
0	0	. 15	. 29	0	. 16
0	0	. 28	0	. 23	. 14
0	0	. 35	0	. 29	. 21
0	0	0	0	. 17	. 16
0	0	0	0	0	. 21
0	0	.19	0	. 20	. 18
0	0	. 24	0	. 35	. 26
. 35	0	. 20	0	. 39	. 15
0	0	. 27	0	0	. 22
0	0	. 20	0	. 8	. 4
0	0	0	0	. 21	. 15
0	0	. 21	0	* 8	. 5
0	0	0	0	. 21	. 14
0	0	. 18	0	. 22	. 21
0	0	. 23	0	* 35	. 27
0	. 30	. 13	0	* 16	. 13
0	0	. 20	0	. 34	. 21
0	. 28	. 14	0	. 14	. 18
0	. 27	. 21	0	. 31	. 24

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In Table IX, 70 species were treated and 62 of the number grew. The following 8 made no growth:

Carya aquatica Nutt., North Amer.
Carya porcina Nutt., North Amer.
Juglans regia L., Europe and Asia.
Liriodendron tulipifera L., North Amer.
Prunus Persica Stokes, Asia.
Quercus alba L., North Amer.
Quercus coccinea Wang., North Amer.
Rhododendron ponticum, var. *Cunninghami* L., Southern Europe and Asia Minor.

A summary of the 70 species tested, in the table below, affords some striking comparisons of results secured from the 16 different treatments.

TABLE X.

Summary of Table IX. Seventy species; treated December 17-24, 1905.

TREATMENT.	Average length of time required for beginning growth.	Average length of time required for buds to open fully.	Percentage that grew.	Percentage that opened their buds fully.
	Days.	Days.		
1 Control	21.5	28.1	58.5	44.2
2 Etherized 48 hours	13.1	20.3	62.8	50.0
3 Etherized 48 + 48 hours	12.7	18.3	47.1	35.7
4 Dried 1 day	20.7	26.2	52.8	45.7
5 Dried 2 days	20.7	25.5	51.4	32.8
6 Dried 3 days	18.0	22.9	47.1	34.2
7 Dried 4 days	19.0	24.4	37.1	28.5
8 Dried 5 days	13.8	18.7	35.7	32.8
9 Frozen 8 days	18.3	23.8	47.1	22.8
10 Frozen 14 days	16.4	23.8	14.2	11.4
11 Dark chamber 8 days	22.8	29.1	55.4	34.2
12 Dark chamber 14 days	23.9	29.2	58.5	32.8
13 Frozen 8 days; etherized 48 hours	11.5	15.5	31.4	15.7
14 Frozen 8 days; etherized 72 hours	9.9	16.4	30.0	21.4
15 Frozen 8 days; dark chamber 5 days	17.5	25.0	21.1	20.0
16 Dark chamber 8 days; etherized 48 hours	20.5	26.5	65.7	38.5
17 Dark chamber 8 days; etherized 72 hours	18.2	25.8	48.5	35.7

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Freezing for 8 days, followed by etherizing for 72 hours caused the quickest average growth, 9.9 days, a gain of 11.6 days over the control.

Severe drying (for 5 days) was as effective as etherizing for 48 hours.

Freezing for 8 days produced a quicker growth than drying for one or two days.

The highest percentage of growth followed the use of the dark-chamber for 8 days, then etherizing for 48 hours, a gain of 7.2 per cent over the control.

The growth was slowest following the use of the dark-chamber alone, loss of one to two days.

Results from drying are interesting. The longer they were dried the smaller the percentage that grew; and, with one exception, the longer they were dried the quicker they grew.

All things considered, etherizing for 48 hours gave the best results.

The value of the methods of treatment for forcing in the order of their worth runs as follows: First, ether (time 12.9 days); second, freezing (time 17.3 days); third, drying (time 18.4 days); and fourth, dark-chamber (time 23.3 days).

The following summary of growth by periods is similar in arrangement and for the same purpose as those given for the other main experiments. Taken in connection with Table X, which is a summary of this last and most important experiment, the following review will prove very interesting and instructive:

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TABLE XI.

Summary of Tables IX and X, showing the extent of growth at the end of 1 wk., 2 wks., 3 wks., and, after 3 weeks.

TREATMENT.	Percentage that began growth in				Percentage that opened their buds fully in			
	1 week.	2 weeks.	3 weeks.	More than 3 weeks.	1 week.	2 weeks.	3 weeks.	More than 3 weeks.
Control	0	24.39	26.83	48.78	0	9.68	16.12	74.17
Etherised 48 hours	24.99	40.90	22.72	11.36	2.86	22.86	28.57	45.71
Etherised 48 + 48 hours	30.30	39.39	18.18	12.12	12.0	16.0	48.00	24.00
Dried 1 day	5.26	23.68	34.20	36.83	3.03	3.03	30.30	63.63
Dried 2 days	2.78	24.99	36.10	36.10	0	4.35	39.12	56.51
Dried 3 days	0	37.14	45.71	17.14	0	0	40.00	60.00
Dried 4 days	4.00	32.0	36.00	28.00	0	10.00	35.00	55.00
Dried 5 days	0	68.00	28.00	4.00	0	9.09	40.91	49.99
Frozen 8 days	3.70	33.30	29.62	33.33	0	5.88	35.29	58.82
Frozen 14 days	0	9.09	90.91	0	0	12.50	12.50	75.00
In dark chamber 8 days	7.50	2.50	40.00	50.00	0	4.00	8.00	88.00
In dark chamber 14 days	4.76	2.38	35.71	57.12	0	4.00	4.00	92.00
Frozen 8 days; etherised 48 hours	36.36	36.36	18.18	9.09	9.09	36.36	36.36	18.18
Frozen 8 days; etherised 72 hours	38.09	47.61	4.76	9.52	0	52.23	26.11	19.99
Frozen 8 days; dark chamber 5 days	0	50.00	15.00	35.00	0	13.33	26.66	59.99
In dark chamber 8 days; etherised 48 hours	4.35	13.04	47.91	34.77	0	0	33.33	66.66
In dark chamber 8 days; etherised 72 hours	6.25	18.75	46.87	28.12	0	0	33.33	66.66

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Those frozen 8 days and then etherized 72 hours show the largest percentage beginning to grow inside of 7 days—38 per cent. Reference to Table X shows this to have been the treatment that caused growth in the shortest average length of time. A further analysis of the results of this treatment reveals the fact that not only did a relatively large number of the species start into growth within the first few days, but the growth continued until the buds were fully open. With one exception, a smaller number remained unopened after three weeks than followed any other treatment. In other words, after 8 days of freezing and 72 hours etherization, four-fifths of the buds were open inside of 22 days, while only one-fourth of the controls were out. Were it necessary, many other comparisons might be made, showing in detail the effects of the different treatments, particularly the results following the use of the ether or freezing alone and in combination.

5. *Miscellaneous Forcing Experiments.*

Time of treatment from January 13 to January 24, 1906.

A few species were found to force with much difficulty or not at all. A list of these were given some special treatments consisting of ether acting for long periods, injecting with 2 per cent sugar solution, and also with distilled water, under pressure of two atmospheres, and by drying. The results follow:

Aesculus flava: etherized for 96 hours, no growth;
etherized for 72+72 hours, began growth in 30 days; leaves fully open in 35 days;
sugar solution, infiltrated for 2 days, no growth;
water, infiltrated for 1 day, grew in 27 days, but leaves never opened;
dried 1 day, grew in 23 days, but leaves did not open;
dried 2 days, grew in 24 days, but leaves did not open;
dried 4 days, grew in 22 days, leaves opened after 30 days;
dried 5 days, grew in 21 days, leaves opened after 30 days;
dried 6 and 7 days, no growth;
dried 8 days, grew in 22 days, leaves open in 34 days;
control, began to grow in 38 days, but leaves did not open.

Alnus viridis: no growth from etherizing 96 and 72+72 hours, or from injections;
dried 1 day, grew in 20 days, leaves open in 28 days;
dried 2 days, no growth;
dried 3 days, grew in 36 days, leaves open in 46 days;
control, no growth.

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Carya aquatica and *C. porcina*: no growth from any of the treatments.

Crataegus macracantha: from etherizing no growth resulted; sugar solution injected, grew in 10 days, leaves open in 28 days;
water injected, grew in 11 days, leaves open in 26 days;
drying, up to 4 days, caused no growth;
control, began to grow in 21 days, but leaves did not open.

Fagus sylvatica: made no growth from any of the treatments but the control grew slightly after 38 days.

Fraxinus excelsior: sugar solution, grew in 23 days; leaves open in 28 days;
water injected, grew in 27 days, leaves open in 35 days;
dried 4 days, slight growth;
control, no growth.

Fraxinus Ornus: a slight growth in 27 days from those that were dried 4 days; no growth from any of the other treatments;
control, no growth.

Juglans regia and *Liriodendron tulipifera*: no growth whatever.

Maclura tinctora: only treated with ether, no growth.
control grew in 6 days, leaves open in 11 days.

Quercus alba and *Q. coccinea*: received all the treatments but made absolutely no growth;
control, no growth.

Quercus pedunculata: etherizing, no growth;
sugar solution, slight growth in flower buds in 9 days, but never opened;

Quercus pedunculata: water injected, slight growth in flower buds, did not open;
dried 1 day, leaf buds grew slightly in 8 days, never opened;
dried 2 days, leaf buds grew slightly in 7 days, but never opened;
dried 3 days, leaf buds grew slightly in 6 days, but did not open;
dried 4 days, leaf buds grew slightly in 12 days, never opened;
dried 5 days, no growth;
control, grew in 19 days, leaves open in 29 days.

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Quercus rubra: etherized, no growth;
sugar solution, grew in 9 days, but did not open;
water injected, grew in 8 days, opened in 12 days;
dried 1 day, grew in 4 days, opened in 9 days;
dried 2 days, grew in 3 days, opened in 11 days;
dried 3 and 4 days, no growth;
control, grew in 19 days, and opened in 23 days.

None of the fourteen species made any growth where etherized for 96 hours and only one, *Aesculus flava*, grew from the 72+72 hours treatment. It appears that at this date, with size of dose used and at the given temperature, the limit of endurance to ether is somewhere between *three and four days* continuous exposure, or between *48+48 and 72+72 hours* of exposing alternately to air and to ether. However, care must be exercised in making comparisons between effects in January and those secured in December. Little was done toward trying to force easily reacting plants in January or later, but it was distinctly observed that the difficult species to force were killed in January and February much quicker than in November and December. In January and February there was far more sunlight and it was much more intense than in the two preceding months and of course the mid-day temperature of the greenhouse was often abnormally high for a short time. The consequent increase in transpiration might account for the quicker death of the twigs, as those having no treatment died as quickly as those that had. This may also serve as an explanation why ether is seemingly more active the higher the temperature is when treated, for then there is a sort of two-fold action—a drying process (loss of water by excessive transpiration), and the action of the ether, whatever that may be. On page 70 the data show that the severest treatment out of many was freezing followed by etherization; and freezing is shown by Mez, Molisch and others to not only remove water from the cells but to force it entirely out of the plant. These two conditions—transpiration and freezing—then, are analogous in so far as both deprive the plant of moisture.

When the sugar solution was used four species grew: *Crataegus macracantha, Fraxinus excelsior, Quercus pedunculata,* and *Q. rubra.*

From the pure water injected five kinds grew: *Aesculus flava, Crataegus macracantha, Fraxinus excelsior, Quercus pedunculata,* and *Q. rubra.*

From drying five species grew: *Aesculus flava, Alnus viridis, Fraxinus excelsior, F. Ornus, Quercus pedunculata* and *Q. rubra.*

Six species made no growth at all, naturally or from any of the treatments: *Carya aquatica, C. porcina, Juglans regia, Liriodendron tulipifera, Quercus alba,* and *Q. coccinea.*

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On January 19, 1906, the following four species were treated:

Amorpha fruticosa: etherized 24 hours, no growth;
control, no growth.

Cornus Mas: etherized 24 hours, grew in 9 days; leaves open in 12 days;
control, grew in 1 day, flowers open in 2 days; no leaves.

Fagus sylvatica: no growth from the control or the etherized specimens.

Forsythia suspensa: etherized 24 hours, grew in 7 days; leaves open in 7 days;
control, flower buds grew in 2 days, open in 6 days; leaf buds grew in 10 days; open in 12 days.

Here, as was the case when treated in November, *Cornus Mas* made no flower growth when ether was used.

On January 29th aand 30th, 1906, ten species were given the following treatments: Chloroform bath, that is, twigs were placed in a glass tube and shaken up with strong chloroform for a few minutes; chloroform gas (25 grams per 100 liters of space); making a smooth longitudinal slit with a razor through all the bud scales and into the center of the buds; and a well lighted moist chamber made by inverting a large glass jar over the plants in the greenhouse.

The following grew after treatment with the chloroform bath: *Alnus viridis* and *Liriodendron* half opened their buds in two days but grew no farther; this was repeated with half strength with the same results. None grew after treatment with the chloroform gas for forty-four hours.

Cutting the buds resulted as follows: *Aesculus flava* grew in thirteen days, leaves opened after twenty-two days; *Alnus viridis,* both leaves and flowers grew in sixteen days; open after twenty-three days; *Fagus sylvatica* began to grow in twenty-nine days; leaves opened fully after fifty-five days. *Fraxinus Ornus* made a noticeable growth in forty-five days but buds did not open.

Following the moist chamber treatment *Alnus viridis* grew in thirty days but did not open; *Fagus sylvatica* grew in thirty-two days but did not open; *Prunus Persica* grew in four days, opening its flowers in nine days; *Rhododendron ponticum,* var. *Cunninghami* grew in thirty days; buds opened in thirty-eight days.

With the control there was a slight growth in *Aesculus flava* in thirty-one days; good growth in *Amorpha fruticosa* in twenty days; buds open in thirty-two days; and a slight growth of *Prunus Persica* although buds did not open. *Juglans regia, Quercus alba,* and *Q. coccinea* did not grow at all.

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In this test *Fagus sylvatica* made a good growth, opening its leaves fully (March 25), for the first time. *Prunus Persica* also grew for the first time.

The effects of wounding the buds (slitting with the razor) were apparent on four of the species. This treatment was in the nature of a mechanical assistance to the buds in opening. It was noticeble with the beech buds that they seemed to be under an internal tension as the wounds yawned open as soon as made.

At different times from February 10 to 17, *Fagus sylvatica* was treated by etherizing for sixteen, twenty-three and twenty-four hours and with half the usual quantity of ether for six hours; slitting the buds and carefully removing all of the scales from the buds, but in no case—controls as well—was there any growth beyond a half-opening of the buds.

6. *A Few Special Treatments.*

Early in the winter several isolated experiments were tried with a few species and some interesting results secured. The first of these tests was with *Cornus Mas* and *Forsythia suspensa.* From November 9 to 14, 1905, *Cornus Mas* was treated with the following results:

Etherized 24 hours, first growth in 14 days, leaves fully open in 36 days.

Etherized 48 hours, first growth in 21 days, leaves fully open in 27 days.

Etherized 72 hours, first growth in 8 days, leaves fully open in 15 days.

Etherized 96 hours, first growth in 9 days, leaves fully open in 13 days.

Etherized 120 hours, first growth in 13 days, leaves fully open in 38 days.

Etherized 144 hours, first growth in 9 days, leaves fully open in 17 days.

Control, first growth in 23 days, flowers fully open in 24 days.

The first four made a vigorous growth, while the next two (120 and 144 hours) were very weak. Not a single flower bud in all those etherized showed any signs of growth and with the control, in this case, there was no leaf growth although the plants were kept until they died. One remarkable feature of this test was the long exposure to ether with growth resulting. Two months later, it is highly probable that such treatment would have killed them speedily.

From November 15 to December 3, *Cornus Mas* was desiccated by submerging twigs in alcohol of strength of 85 to 95 per cent, and in a saturated solution of common salt; also dried in a desiccator over concentrated sulphuric acid, and in a warm room for different periods of time. The results follow:

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In alcohol 3½ hours, first growth in 9 days; flowers fully open in 16 days;

In alcohol 23 hours or in NaCl 7½ and 23 hours, no growth;

In desiccator 15 hours, grew in 7 days, flowers fully open in 9 days;

Dried 1 day, loss of weight 11.6%, grew in 6 days; flowers open in 7 days;

Dried 2 days, loss of weight 17%, grew in 7 days but did not open;

In alcohol 23 hours or in NaCl 7½ and 23 hours, no growth;

Dried 4 days, loss of weight 27%, no growth;

Dried 5 days, loss of weight 30.40%, no growth;

Dried 6 days, loss of weight 32.03%, grew in 12 days, leaves did not open;

Dried 7 days, loss of weight 30.96%, no growth;

Dried 8 days, loss of weight 37%, no growth;

Control, first growth in 7 days, flowers open in 8 days.

These figures would seem to indicate that *C. Mas* cannot withstand strong desiccation, but in still another trial the results are somewhat different. Here also the twigs were dried in a warm room; dates, November 16 to December 3, 1905.

Dried 1 day, loss of weight 7.89%, grew in 10 days, flowers open in 11 days;

Dried 2 days, loss of weight 14.28%, grew in 6 days, flowers open in 7 days;

Dried 3 days, loss of weight 18.88%, no growth;

Dried 4 days, loss of weight 21.73%, grew in 7 days, flowers open in 8 days;

Dried 5 days, loss of weight 23.68%, grew in 9 days, flowers open in 10 days.

Dried 6 days, loss of weight 27.34%, grew in 10 days but did not open;

Dried 7 days, loss of weight 25.60%, no growth;

Dried 8 days, loss of weight 20.00%, no growth;

Dried 9 days, loss of weight 22.41%, no growth;

Dried 10 days, loss of weight 31.06%, no growth;

Control, first growth in 23 days, flowers open in 24 days.

In this test drying, up to a certain point, usually gave good results in forcing flower growth, but with this species no flowers have been secured following the use of ether. While ether and desiccation produced the same general results, that is, growth, the particular effects of the two on the flower and leaf buds in *C. Mas* are very different: The drying very rarely produced a leaf, while the ether never produced a flower.

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Forsythia suspensa was etherized and desiccated with alcohol and salt solution November 16 to 18, 1905, with the following results:

Etherized 24 hours, grew in 2 days, flowers open in 9 days;

Etherized 48 hours, grew in 3 days, flowers open in 10 days;

In alcohol 22 hours, and in NaCl sol. 22 hours, no growth from either;

Control, first growth in 7 days, flowers open in 13 days, but was really not in full bloom until 7 days later, or 22 days from date placed in greenhouse, while those etherized for 24 hours were in gorgeous full bloom in 9 days from date of treatment.

Other tests with *Forsythia* (not given here) gave similar results. It is injured by 48 hours of ether. On account of the ease, quickness and perfection with which the *Forsythia* is forced in early and mid-winter should make it useful in a commercial way.

Aesculus Hippocastanum treated by air drying from November 25 to December 3, 1905, gave these results;

Dried 1 day, loss of weight 3.30%, grew in 18 days, leaves open in 26 days;

Dried 2 days, loss of weight 3%, grew in 16 days, leaves open in 27 days;

Dried 3 days, loss of weight 3.26%, grew in 15 days, leaves open in 27 days;

Dried 4 days, loss of weight 6.63%, grew in 13 days, leaves open in 18 days;

Dried 5 days, loss of weight 8.67%, grew in 12 days, leaves open in 17 days;

Dried 6 days, loss of weight 10.52%, grew in 10 days, leaves open in 16 days;

Dried 7 days, loss of weight 11%, grew in 26 days, leaves open in 31 days;

Dried 8 days, loss of weight 10.48%, grew in 21 days, leaves open in 28 days;

Control, grew in 20 days and opened its leaves in 31 days.

The results are interesting in that up to 6 days the more they were dried the quicker the growth began. *Castanea pumila* similarly treated at the same time made no growth at all.

Castanea vesca made a full growth in 15 to 16 days when dried 5, 6 and 7 days, with loss of weight of from 19 to 23 per cent; the control made a slightly better growth at the end of 21 days. *Gleditschia triacanthos* made no growth when dried up to 9 days, and *Robinia Pseud-acacia* grew very slightly when dried 1 day, loss of weight 6.32%, growth in 20 days; and when dried 7 days—loss of weight 21.79%—grew in 13 days.

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EXPERIMENTS WITH POTTED PLANTS.

Of much more interest were the developments from treating a few specimens of *Quercus pedunculata* which had been growing in pots for two years, or from the time they came from the seed. During late autumn and up to the time the experiment began, the pots were kept in a coldframe in the garden where they were slightly protected with board covers, but had absolutely no artificial heat. The weather record (Table XII, pp. 83-4) shows that on the 20th and 21st of October there were freezing temperatures and again on the 12th, 17th, 18th, 19th, and 21st of November the outside temperature was below zero Centigrade. It is highly probable that after once becoming frozen they did not thaw out again as the bed was in the shade and not much influenced by the outside temperature. When removed for treatment on November 25 the soil in the pots was frozen quite hard. For a day the plants were kept in a cool basement room at about 10° C. in order that they might thaw out gradually. Between November 27 and December 9 all were treated with ether and artificial freezing, one plant being used for each treatment. The results follow:

November 28, etherized 24 hours, leaves fully open 30 days.
November 27, etherized 28 hours, leaves fully open 24 days.
December 1, etherized 48+48 hours, leaves fully open **14** days.
December 9, frozen 7 hours, leaves fully open 34 days.
November 27, control, leaves fully open **31** days.

According to the results, growth was accelerated in direct proportion to the increase of the ether, the greatest acceleration being 17 days. Strong freezing actually retarded the growth and it should be added that the plant was so badly injured that it died within a few days after the leaves were out.

The most astonishing feature about this case was that the oaks should have grown at all, especially the control, as similar plants of the same species in the same room made absolutely no signs of growth when kept there all winter without treatment. These plants, however, had always been kept in this greenhouse during the two years of their life, but they still go dormant in autumn and remain so until spring.

The explanation of the behavior of the treated oaks, it is believed, lies in the fact of their having been frozen before treatment and sufficiently so to cause growth. The ether, however, accelerated the growth in very marked degree.

All three of the etherized oaks, together with the control, made a luxuriant leaf growth (the treated ones being somewhat better) forming branches and increasing the length of the main stem. An-

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other note-worthy feature of their further behavior was, that after forming from 12 to 20 full grown leaves, all began to show signs of slow growth. All but one were now removed to a cool greenhouse where the day temperature was between 7° and 10° C. and the humidity 80 to 85 per cent. Here they soon ceased to grow entirely and during the first week in March were observed to have developed strong terminal buds. But the specimen remaining in the warm greenhouse exhibited the same phenomenon of forming a terminal bud, although slightly later than the others. This plant was now also removed to the cool room.

The first week in May the control specimen and the one that had been etherized 48 hours began to grow again and a week later the one etherized 48+48 hours also grew. The remaining plant, which had had 24 hours of ether, was nearly two weeks later still, in beginning growth. The first three began to grow at about the same time as some others in pots that had been in the cool greenhouse all winter.

From December 18 to 24 more of the *Quercus pedunculata* in pots were treated as follows:

1. Etherized 72 hours, leaves were fully open after 23 days.
2. Frozen 5 days, leaves were fully open after 67 days.
3. In dark-chamber 20 days, leaves were fully open after 30 days.
4. Control, leaves were fully open after 23 days.

These plants were under the same conditions before treatment as those in the preceding test, that is, in a coldframe in the garden and, of course, had been frozen. Indeed, the last days of November and first week of December were colder than early in November (see Table XII) and hence they had been frozen for about a month, which may account for the slight effects of the treatment. As the data show, none of the treatments hastened the growth at all and the freezing and dark-chamber greatly retarded it, the freezing especially being injurious in this respect.

The first, third and fourth of the above ceased growing and formed resting buds in March but the second had at that time only begun to grow and that very feebly. In fact the growth that did occur came from a sprout which grew out from the base of the plant.

Mediterranean Plants.—Twigs of a number of Mediterranean species were taken from plants kept in a cool plant house and placed in the warm greenhouse November 18, 1905, but were not treated.

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They remained in fresh water until all died (some living until March), but there were no signs of growth in any of the species. The following is the list:

Buxus balearica Lam.
Ceratonia Siliqua L.
Citrus vulgaris Risso.
Ficus Carica L.
Laurus nobilis L.
Myrtus communis L.
Nerium oleander L.
Olea europaea L.
Phillyrea latifolia L.
Pistacia vera L.
Prunus Pseudo-Suber L.
Viburnum Tinus L.

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TABLE XII.

DAILY TEMPERATURES DURING THE WINTER OF 1905-6.

From the Weather Bureau of the Agricultural Institute of the University of Halle.

PROF. DR. HOLDEFLEISS, *Observer.*

* = Hoar-frost.

Date.	OCTOBER.			NOVEMBER.			DECEMBER.			JANUARY.			FEBRUARY.		
	Temperature Cent.			Temperature Cent.			Temperature Cent.			Temperature Cent.			Temperature Cent.		
	Max.	Min.	Mean.	Max.	Min.	Mean.	Max.	Min.	Mean.	Max.	Min.	Mean.	Max.	Min.	Mean.
1	12.7	6.8	9.0	14.2	6.5	9.0	1.5	—1.0°	0.7	—3.9	—9.7	—5.9	3.1	—0.9°	1.6
2	11.4	7.3	9.0	10.4	2.2	6.0	2.0	—0.4	0.0	—3.5	—8.2	—7.0	4.2	2.2	2.9
3	11.4	6.7	7.8	12.0	6.0	8.4	0.2	—2.2	—1.2	—1.9	—9.0	—5.0	3.5	0.4	1.2
4	11.6	4.5	9.3	12.2	4.7	7.0	—0.6	—2.4°	—0.1	4.5	—5.2	2.2	1.9	—1.2	—0.1
5	13.6	7.2	9.4	11.8	4.2	8.8	0.8	—0.9	0.0	8.8	2.5	5.9	0.4	—1.4	—0.5
6	10.8	6.5	7.6	12.7	5.5	8.4	2.4	—1.1	1.1	7.0	4.8	6.6	0.2	—1.2	—0.9
7	9.6	5.6	6.9	9.9	0.6°	4.9	6.5	1.8	5.1	8.0	8.2	5.0	—0.1	—1.1	—0.6
8	9.5	5.8	7.8	7.8	4.1	6.0	11.4	6.2	10.4	5.3	1.4	3.7	2.8	—0.8	1.2
9	8.2	3.8	6.6	6.7	5.1	5.6	11.2	4.0	5.6	5.5	1.2	3.2	1.2	—1.5	—0.8
10	8.5	5.0	6.3	6.0	3.7	4.0	6.9	1.9	4.3	7.3	3.3	4.4	2.5	—2.4	—0.8
11	9.4	2.6°	6.6	5.6	1.4	2.6	4.8	0.9	2.5	6.2	1.8	3.3	3.6	—1.6	1.0
12	8.6	5.5	7.0	4.8	—0.4°	1.6	3.0	—0.2	1.7	5.0	1.1°	3.5	4.4	—2.1	0.4
13	9.5	4.4	6.4	4.1	1.2	2.9	4.3	0.7	3.2	9.3	3.2	7.3	0.2	—2.5	—1.5
14	7.5	1.7	5.0	3.7	2.0	2.4	7.2	3.9	6.1	8.6	3.1	4.1	0.7	—3.5	—0.6
15	9.0	3.8	5.9	3.5	0.2	1.2	7.1	5.3	6.0	6.3	1.4°	3.4	4.0	—0.5	1.6
16	9.6	1.3	4.5	1.4	—0.2	0.2	6.4	4.3	5.1	3.7	1.4	5.0	5.3	—0.2	1.6
17	9.0	3.4	4.9	2.2	—0.7	—0.0	5.5°	—0.3	0.6	10.1	1.3	3.7	7.5	—1.4	3.0

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TABLE XII. (*Continued.*)

DAILY TEMPERATURES DURING THE WINTER OF 1905-6.

From the Weather Bureau of the Agricultural Institute of the University of Halle,

PROF. DR. HOLDEFLEISS, *Observer.*

* = Hoar-frost.

Date.	OCTOBER.			NOVEMBER.			DECEMBER.			JANUARY.			FEBRUARY.		
	Temperature Cent.			Temperature Cent.			Temperature Cent.			Temperature Cent.			Temperature Cent.		
	Max.	Min.	Mean.	Max.	Min.	Mean.	Max.	Min.	Mean.	Max.	Min.	Mean.	Max.	Min.	Mean.
18	7.9	1.6*	3.8	2.1	—2.2*	—0.9	—0.2*	—3.6	—3.2	9.6	1.5	6.6	5.7	2.4	3.9
19	6.9	1.5*	3.2	1.6	—2.3*	—0.4	0.9*	—4.9	—1.8	9.8	1.8	3.5	4.5	2.1	3.0
20	6.0	—1.0*	1.1	2.2	0.4	1.2	2.8	—1.8	1.7	4.0	0.7	1.8	5.7	2.2	3.7
21	7.2	—1.9*	2.8	5.5	—1.1	2.6	4.2	2.0	3.6	4.2	1.3	2.0	5.8	1.6	3.2
22	5.6	1.0	3.1	3.4	1.7*	1.0	6.8	3.7	5.6	1.8	—3.9	—2.4	5.6	0.8	2.2
23	6.1	0.1*	2.7	3.3	0.3*	1.7	6.5	4.3	5.2	—2.1	—5.7	—4.4	6.0	0.6	2.8
24	4.3	0.2	1.2	5.5	1.6	3.3	4.6	2.3	2.6	—1.6	—5.9	—3.1	3.3	—0.1	0.9
25	4.0	—1.2*	1.2	6.3	2.1	3.4	3.0	—0.3	0.5	—1.7	—8.6	—4.5	4.5	—1.0*	2.2
26	5.5	0.4	2.0	8.7	0.9*	6.4	2.0	—0.7	1.4	2.3	—4.4	1.0	6.9	2.0	4.6
27	3.8	—0.6	2.5	11.7	4.4	6.9	5.5	1.2	2.3	6.7	2.0	4.5	9.5	4.4	7.8
28	8.4	2.1	5.2	7.0	2.2	3.5	1.7*	—2.6	—1.2	6.8	4.2	5.1	8.3	3.2	4.8
29	9.0	3.8	6.0	4.5	—0.1*	1.2	5.0	—0.4	3.8	4.9	2.8	4.1	....	....	....
30	12.4	5.3	9.3	0.5	—2.8*	—1.2	5.5	—4.8	—2.5	6.0	3.2	4.1	....	....	....
31	13.0	8.3	9.8	....	....	....	—3.3	—6.3	—4.6	4.4	1.0	1.7	....	....	....
Monthly Sum.	270.0	102.0	173.4	190.8	48.3	108.5	126.8	7.6	64.5	148.2	—12.4	63.4	111.2	—1.5	48.0
Monthly Mean.	8.7	3.3	5.6	6.4	1.6	3.6	4.1	0.2	2.1	4.8	—0.4	2.0	4.0	—0.1	1.7

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EXPERIMENTS DURING THE WINTER OF 1906-7.

(Tables XIII, XIV and XV.)

These experiments were carried on in the Horticultural Department of the University of Missouri during the fall of 1906 and the winter of 1907. In these tests three objects were sought:

First, to compare the behavior of plants grown here with similar species grown in central Europe,

Second, to test the effect of time of year—that is, the date when treatment was given, on the number of days required for growth to begin; and,

Third, to find whether dormant plants while being etherized give off more moisture than plants under similar conditions untreated.

Only a short list of species was used as it was desired only to test a few typical forms that had been found to force easily, such as the *Forsythia,* to force with moderate ease, as the *Acer, Platanus* and *Castanea,* and some that grew with great difficulty, like the *Fraxinus* and *Quercus alba.* Unfortunately it was not practicable to secure *Fagus* and *Liriodendron* in sufficient quantity to include them in the experiments.

Only two general treatments were employed—etherizing and air drying in a warm, dry, laboratory room. By omitting many of the combination treatments the number of plants to be handled was sufficiently reduced to make it possible to treat a new set every ten days from late fall until the close of winter. The same methods of treatment were followed in these tests as were employed the previous winter. Also the same quantity of ether was used as before, that is, forty grams per one hundred liters of space.

The treatments began on October 30, 1906, and were repeated every ten days until February 12, 1907, thus covering a period of ninety days. Thirteen species were used. In Table XIII which follows will be seen the results of the treatments in detail, arranged in groups according to date of treatment:

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TABLE XIII.

Thirteen species treated on different dates by etherizing and by drying during the fall and winter of 1906-7.

1 Treated between Oct. 30 and Nov. 10, 1906.	Control.	Etherized 24 hours.	Etherized 24 + 24 hours.	Etherized 48 hours.	Etherized 48 + 48 hours.	Dried 1 day.	Dried 2 days.	Dried 3 days.	Dried 4 days.	Dried 5 days.	Dried 6 days.	Dried 7 days.	Dried 8 days.	Dried 9 days.	Dried 10 days.
	Growth began in	Growth began in	Growth began in	Growth began in	Growth began in	Growth began in	Growth began in	Growth began in	Growth began in	Growth began in	Growth began in	Growth began in	Growth began in	Growth began in	Growth began in
	days	days	days	days	days	days	days	days	days	days	days	days	days	days	days
Acer platanoides L.	0	0	—	—	—	0	0	0	0	0	0	0	0	0	0
Acer rubrum L.	0	0	—	—	—	0	0	0	0	0	0	0	0	0	0
Acer saccharinum Wang.	0	0	—	—	—	0	0	0	0	0	0	0	0	0	0
Castanea sativa, var. americana Watson	0	0	—	—	—	0	0	0	0	0	0	0	0	0	0
Forsythia suspensa Vahl.	11	17	—	—	—	0	0	0	0	0	—	—	—	—	—
Fraxinus viridis Michx.	0	0	—	—	—	0	0	0	0	0	0	0	0	0	0
Gleditschia triacanthos L.	0	0	—	—	—	0	0	0	0	0	0	0	0	0	0
Platanus occidentalis L.	0	0	—	—	—	0	0	0	0	0	0	0	0	0	0
Quercus alba L.	0	0	—	—	—	0	0	0	0	0	0	0	0	0	0
Quercus Muehlenbergii Engelm.	0	0	—	—	—	0	0	0	0	0	0	0	0	0	0
Taxodium distichum Rich.	0	56	—	—	—	0	0	0	0	0	0	0	0	0	0
Thuja occidentalis L.	0	0	—	—	—	0	0	0	0	0	0	0	0	0	0
Ulmus americana L.	0	0	—	—	—	0	0	0	0	0	0	0	0	0	0

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TABLE XIII. (*Continued.*)

2

Treated between Nov. 18 and 23, 1906.

Acer platanoides L.	0	0	—	—	—	0	0	0	0	0	0	0	29	28	21
Acer rubrum L.	0	0	—	—	—	0	0	0	0	26	16	0	0	0	0
Acer saccharinum Wang.	0	0	—	—	—	0	0	0	0	0	0	0	0	0	0
Castanea sativa, var. americana Watson	0	0	—	—	—	0	0	0	0	0	0	0	0	0	0
Forsythia suspensa Vahl.	15	10	—	—	—	22	0	23	31	0	—	—	—	—	—
Fraxinus viridis Michx.	48	61	—	—	—	0	55	47	48	48	0	21	44	22	15
Gleditschia triacanthos L.	0	0	—	—	—	0	0	0	0	0	0	0	0	0	0
Platanus occidentalis L.	0	0	—	—	—	0	0	0	0	0	0	0	0	0	0
Quercus alba L.	0	0	—	—	—	0	0	0	0	0	0	0	0	0	0
Quercus Muehlenbergii Englm.	0	40	—	—	—	44	0	0	0	0	0	0	0	0	0
Taxodium distichum Rich.	37	0	—	—	—	0	0	0	41	0	0	0	0	0	0
Thuja occidentalis L.	43	0	—	—	—	0	0	0	27	0	0	0	0	0	0
Ulmus americana L.	37	44	—	—	—	44	43	0	0	0	0	0	0	0	0

3

Treated between Nov. 24 and Dec. 4, 1906.

Acer platanoides L.	0	—	0	0	10	0	18	0	0	0	0	0	0	0	0
Acer rubrum L.	0	—	0	0	14	0	0	0	0	0	0	0	0	0	0
Acer saccharinum Wang.	0	—	0	0	0	0	0	0	0	0	0	0	0	0	0
Castanea sativa, var. americana Watson	0	—	0	0	33	0	0	0	0	0	0	0	0	0	0
Forsythia suspensa Vahl.	11	10	4	—	—	23	16	21	12	11	—	—	—	—	—
Fraxinus viridis Michx.	0	—	34	17	0	23	26	0	0	38	31	0	36	30	0
Gleditschia triacanthos L.	0	—	0	0	0	0	0	0	0	0	0	0	0	0	0
Platanus occidentalis L.	0	—	0	0	0	0	0	0	0	0	0	0	0	28	0
Quercus alba L.	0	—	0	0	0	0	0	0	0	0	0	0	0	0	0
Quercus Muehlenbergii Engelm.	0	—	0	0	0	0	0	0	0	0	0	0	0	0	0
Taxodium distichum Rich.	30	—	29	0	0	0	0	0	0	0	0	0	0	0	0
Thuja occidentalis L.	0	—	17	18	0	0	0	0	0	26	0	0	0	0	0
Ulmus americana L.	0	—	13	0	0	0	0	23	0	11	0	0	0	0	0

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TABLE XIII. (*Continued.*)

Thirteen species treated on different dates by etherizing and by drying during the fall and winter of 1906-07.

4 Treated between Dec. 5 and 15, 1906.	Control.	Etherized 24 hours.	Etherized 24 + 24 hours.	Etherized 48 hours.	Etherized 48 + 48 hours.	Dried 1 day.	Dried 2 days.	Dried 3 days.	Dried 4 days.	Dried 5 days.	Dried 6 days.	Dried 7 days.	Dried 8 days.	Dried 9 days.	Dried 10 days.
	Growth began in	Growth began in	Growth began in	Growth began in	Growth began in	Growth began in	Growth began in	Growth began in	Growth began in	Growth began in	Growth began in	Growth began in	Growth began in	Growth began in	Growth began in
	days	days	days	days	days	days	days	days	days	days	days	days	days	days	days
Acer platanoides L.	0	—	0	0	0	0	0	0	0	0	0	0	0	0	0
Acer rubrum L.	0	—	0	45	0	0	0	0	0	0	0	0	0	0	0
Acer saccharinum Wang.	0	—	0	0	0	0	0	0	0	0	0	0	0	0	0
Castanea sativa, var. americana Watson	0	—	0	47	29	0	0	0	0	0	0	0	0	0	0
Forsythia suspensa Vahl.	19	6	12	—	—	14	17	0	9	10	—	—	—	—	—
Fraxinus viridis Michx.	30	—	21	49	15	21	42	0	22	25	0	19	0	13	16
Gleditschia triacanthos L.	0	—	0	0	0	0	0	0	0	0	0	0	0	0	0
Platanus occidentalis L.	0	—	63	61	65	46	0	74	0	0	41	0	0	38	0
Quercus alba L.	0	—	0	47	29	0	28	0	0	0	0	0	0	0	0
Quercus Muehlenbergii Engelm.	0	—	0	0	24	0	52	0	0	0	0	0	0	0	0
Taxodium distichum Rich.	0	—	12	14	13	18	0	0	15	21	0	0	0	0	0
Thuja occidentalis L.	26	—	16	69	0	0	21	68	0	0	0	21	0	0	0
Ulmus americana L.	19	—	0	2	0	48	13	0	19	0	0	0	0	0	0

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TABLE XIII. (*Continued.*)

5

Treated between Dec. 15-26, 1906.

Acer platanoides L.	0	—	0	0	0	0	0	0	0	0	0	0	22	18	0
Acer rubrum L.	0	—	0	0	0	0	0	0	0	0	0	0	26	0	0
Acer saccharinum Wang.	0	—	0	0	0	0	0	0	0	0	0	0	0	0	0
Castanea sativa, var. americana Watson	0	—	0	0	28	0	49	0	0	0	0	0	0	0	0
Forsythia suspensa Vahl.	18	12	8	—	—	18	11	15	9	10	—	—	—	—	—
Fraxinus viridis Michx.	18	—	17	16	48	33	51	34	26	8	7	23	8	0	0
Gleditschia triacanthos L.	0	—	0	31	0	0	0	0	0	0	0	0	0	0	0
Platanus occidentalis L.	0	—	0	70	50	71	74	55	0	51	47	0	0	25	0
Quercus alba L.	0	—	0	35	19	38	0	38	0	0	0	0	0	0	0
Quercus Muehlenbergii Engelm.	41	—	0	31	31	0	0	0	0	0	0	0	0	0	0
Taxodium distichum Rich.	0	—	6	16	5	0	0	0	0	22	0	0	0	0	0
Thuja occidentalis L.	0	—	18	16	0	0	0	0	0	0	22	20	0	0	0
Ulmus americana L.	20	—	0	0	0	0	28	0	9	0	0	4	0	0	0

6

Treated between Dec. 25, 1906 and Jan. 4, 1907.

Acer platanoides L.	0	—	0	0	0	0	0	57	0	52	0	0	0	0	0
Acer rubrum L.	0	—	40	6	7	0	0	0	34	22	0	0	0	0	0
Acer saccharinum Wang.	0	—	0	0	0	0	0	0	0	0	0	0	0	0	0
Castanea sativa, var. americana Watson	0	—	0	0	23	0	0	0	0	0	0	0	0	0	0
Forsythia suspensa Vahl.	13	10	9	—	—	0	13	10	17	16	—	—	—	—	—
Fraxinus viridis Michx.	43	—	19	27	0	26	23	24	23	8	21	10	14	0	0
Gleditschia triacanthos L.	15	—	5	49	0	0	64	0	0	0	0	0	0	0	0
Platanus occidentalis L.	64	—	0	0	51	45	61	0	0	0	56	44	0	0	0
Quercus alba L.	0	—	0	0	0	0	0	0	0	0	0	0	0	0	0
Quercus Muehlenbergii Engelm.	41	—	0	0	0	0	44	24	0	0	0	0	0	0	0
Taxodium distichum Rich.	20	—	10	22	21	21	22	14	0	19	14	17	0	0	0
Thuja occidentalis L.	22	—	17	1	16	19	20	19	16	24	0	20	0	42	0
Ulmus americana L.	8	—	0	0	0	2	6	5	4	12	0	0	0	0	0

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TABLE XIII. (*Continued.*)

Thirteen species treated on different dates by etherizing and by drying during the fall and winter of 1906-07.

7 Treated between Jan. 5-21, 1907.	Control.	Etherized 24 hours.	Etherized 24 + 24 hours.	Etherized 48 hours.	Etherized 48 + 48 hours.	Dried 1 day.	Dried 2 days.	Dried 3 days.	Dried 4 days.	Dried 5 days.	Dried 6 days.	Dried 7 days.	Dried 8 days.	Dried 9 days.	Dried 10 days.
	Growth began in	Growth began in	Growth began in	Growth began in	Growth began in	Growth began in	Growth began in	Growth began in	Growth began in	Growth began in	Growth began in	Growth began in	Growth began in	Growth began in	Growth began in
	days	days	days	days	days	days	days	days	days	days	days	days	days	days	days
Acer platanoides L.	53	—	29	47	14	52	51	0	51	48	0	0	0	0	0
Acer rubrum L.	11	—	27	25	26	0	49	13	54	0	0	0	0	0	0
Acer saccharinum Wang.	0	—	0	0	0	0	0	0	0	0	0	0	0	0	0
Castanea sativa, var. americana Watson	0	—	0	33	21	43	0	41	0	0	0	0	0	0	0
Forsythia suspensa Vahl.	11	15	8	—	—	12	0	0	0	8	—	—	—	—	—
Fraxinus viridis Michx.	32	—	37	38	0	31	42	37	0	13	0	13	30	0	0
Gleditschia triacanthos L.	0	—	9	7	12	48	0	0	0	0	0	0	0	0	0
Platanus occidentalis L.	53	—	43	33	26	45	45	46	49	30	0	0	0	0	0
Quercus alba L.	0	—	0	0	0	0	0	0	0	0	0	0	0	0	0
Quercus Muehlenbergii Engelm.	32	—	0	25	0	48	0	32	0	0	0	0	0	0	0
Taxodium distichum Rich.	16	—	8	9	0	0	14	13	0	8	0	0	0	0	0
Thuja occidentalis L.	0	—	13	0	10	52	0	37	0	41	0	0	0	0	0
Ulmus americana L.	10	—	17	28	0	0	0	0	0	0	0	6	0	0	0

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TABLE XIII. (*Continued.*)

8

Treated between Jan. 21 and Feb. 2, 1907.

Acer platanoides L.	24	—	13	17	13	18	28	30	26	25	0	19	0	0	0
Acer rubrum L.	24	—	0	0	24	34	0	0	0	0	0	0	0	0	0
Acer saccharinum Wang.	0	—	0	17	13	0	0	0	0	0	0	0	0	0	0
Castanea sativa, var. americana Watson	35	—	0	0	20	0	0	0	0	0	0	0	0	0	0
Forsythia suspensa Vahl.	11	10	8	—	—	10	9	8	7	6	—	—	—	—	—
Fraxinus viridis Michx.	21	—	0	0	0	13	35	0	0	0	24	0	0	0	0
Gleditschia triacanthos L.	0	—	27	22	5	34	0	36	0	0	0	0	0	0	0
Platanus occidentalis L.	35	—	0	0	15	34	0	34	33	30	0	19	16	0	0
Quercus alba L.	0	—	36	0	22	0	26	25	0	0	0	0	0	0	0
Quercus Muehlenbergii Engelm.	30	—	21	26	20	0	35	21	0	19	0	0	0	0	0
Taxodium distichum Rich.	4	—	8	33	13	38	0	8	7	0	0	0	0	0	0
Thuja occidentalis L.	21	—	21	17	13	23	24	21	0	0	0	0	0	0	0
Ulmus americana L.	4	—	17	0	0	7	9	8	7	9	0	0	0	0	0

9

Treated between Feb. 2-12, 1907.

Acer platanoides L.	0	—	0	16	8	0	0	0	14	0	0	0	17	0	13
Acer rubrum L.	7	—	4	7	8	6	14	4	0	4	0	11	0	0	0
Acer saccharinum Wang.	0	—	0	0	0	0	0	0	0	0	0	0	0	0	0
Castanea sativa, var. americana Watson	0	—	20	0	17	0	0	18	0	0	0	0	0	0	0
Forsythia suspensa Vahl.	9	6	6	—	—	6	5	9	5	7	—	—	—	—	—
Fraxinus viridis Michx.	7	—	20	10	17	0	23	0	10	0	17	0	15	0	0
Gleditschia triacanthos L.	6	—	4	5	0	0	0	18	0	20	0	0	0	0	0
Platanus occidentalis L.	0	—	22	0	19	0	0	0	0	0	15	18	17	0	0
Quercus alba L.	0	—	0	0	0	0	0	0	0	0	0	0	0	0	0
Quercus Muehlenbergii Engelm.	0	—	22	19	8	26	25	18	14	0	0	0	0	0	0
Taxodium distichum Rich.	14	—	13	0	6	0	0	11	23	18	17	18	0	0	0
Thuja occidentalis L.	16	—	11	12	8	26	16	13	19	18	15	0	0	0	0
Ulmus americana L.	7	—	0	0	1	6	5	0	5	0	0	0	0	0	0

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In the first test only two species grew, the golden bell and the bald cypress.

In the second treatment it is surprising to note the behavior of the green ash which grew from both etherizing and drying and also where not treated. This particular species was not included in any of the tests conducted the year before in Germany, but the white ash, *Fraxinus americana* was, and the two species are very similar in every respect.

The *Fraxinus viridis* grew in every one of the nine tests. In the second experiment the sugar maple, chestnut, honey locust, sycamore and white oak made no growth.

In the third trial the honey locust and white oak still did not grow, and, from some cause, the chinquapin oak failed to start, although it grew in a previous test.

In the fourth test—December 5-15, the *Quercus alba* grew from two of the strong ether treatments and also in one instance from slight drying. It also grew in the next test ten days later, from four of the treatments. The year before, the same species grown in Germany, could not be made to grow from any treatment until almost spring.

In tests six, seven and nine the *Quercus alba* made no growth, but it grew from four treatments in the eighth experiment. In no case, however, did the control specimens make any growth. The entire results of all the tests are summarized in Table XIV.

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TABLE XIV. (Summary of Table XIII.)

Record of 13 species treated at different times by etherizing and drying, showing the average number of days required for growth to begin and the percentage that grew from each treatment.

TREATMENT.	1. Treated between Oct. 30 & Nov. 10, 1906.		2. Treated between Nov. 13 & 23, 1906.		3. Treated between Nov. 24 & Dec. 4, 1906.		4. Treated between Dec. 5 & 15, 1906.		5. Treated between Dec. 15 & 25, 1906.		6. Treated between Dec. 25, '06 & Jan. 4, '07.	
	Av. No. Days for growth to begin	Percentage that grew	Av. No. Days for growth to begin	Percentage that grew	Av. No. Days for growth to begin	Percentage that grew	Av. No. Days for growth to begin	Percentage that grew	Av. No. Days for growth to begin	Percentage that grew	Av. No. Days for growth to begin	Percentage that grew
Control	11.	7.69	36.	38.45	21.5	15.38	28.5	30.76	21.7	30.76	25.7	61.52
Etherized 24 hours.	36.5	15.38	38.7	30.76	10.	7.69	6.	7.69	12.	7.69	10.	7.69
Etherized 24 + 24 hours.	....	....	....	....	19.4	38.45	20.8	38.45	11.	30.76	16.6	46.14
Etherized 48 hours	....	....	....	....	17.5	16.66	41.7	68.64	30.7	58.31	21.2	41.65
Etherized 48 + 48 hours.	....	....	....	....	19.	24.99	29.1	49.98	30.1	49.98	23.6	41.65
Dried 1 day	0	0	36.6	23.07	23.	15.38	29.4	38.45	38.7	30.76	22.6	38.45
Dried 2 days	0	0	49	15.38	20.	23.07	28.8	46.14	42.6	38.45	31.6	61.52
Dried 3 days	0	0	35	15.38	22.	15.38	71.	15.38	35.5	30.76	21.8	53.83
Dried 4 days	0	0	36.7	23.07	12.	7.69	16.2	30.76	14.6	23.07	18.8	38.45
Dried 5 days	0	0	34.5	15.38	21.2	30.76	18.6	23.07	22.7	30.76	21.8	53.83
Dried 6 days	0	0	16	7.69	31.	8.33	41.	8.33	25.3	24.99	30.3	24.99
Dried 7 days	0	0	21	7.69	0	0	20.	16.66	15.6	24.99	22.7	33.32
Dried 8 days	0	0	36.6	15.38	36.	8.33	0	0	18.6	24.99	14.	8.33
Dried 9 days	0	0	25	15.38	29.	16.66	25.5	16.66	21.5	16.66	42.	8.33
Dried 10 days	0	0	18	15.38	0	0	16.	8.33	0	0	0	0

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TABLE XIV. (Summary of Table XIII.) (*Continued.*)

Record of 13 species treated at different times by etherizing and drying, showing the average number of days required for growth to begin and the percentage that grew from each treatment.

TREATMENT.	7. Treated between Jan. 5 & 21, 1907.		8. Treated between Jan. 21 & Feb. 21, 1907.		9. Treated between Feb. 2 & 12, 1907.	
	Av. No. Days for growth to begin	Percentage that grew	Av. No. Days for growth to begin	Percentage that grew	Av. No. Days for growth to begin	Percentage that grew
Control	27.2	61.52	19.9	76.90	9.4	53.83
Etherized 24 hours	15.	7.69	10.	7.69	6.	7.69
Etherized 24 + 24 hours	21.2	69.21	18.8	61.52	13.4	69.21
Etherized 48 hours	27.2	75.07	22.	49.98	11.5	49.98
Etherized 48 + 48 hours	18.1	49.98	15.8	83.30	9.6	75.07
Dried 1 day	41.3	61.52	23.4	69.21	14.	38.45
Dried 2 days	40.2	38.45	23.7	53.83	14.6	46.14
Dried 3 days	31.2	53.83	21.1	69.21	13.	53.83
Dried 4 days	51.3	23.07	16.	38.45	12.8	53.83
Dried 5 days	29.6	46.14	17.8	38.45	13.4	38.45
Dried 6 days	0	0	24.	8.33	16.	33.32
Dried 7 days	9.5	16.66	19.	16.66	15.6	24.99
Dried 8 days	30.	8.33	16.	8.33	16.3	24.99
Dried 9 days	0	0	0	0	0	0
Dried 10 days	0	0	0	0	13.	8.33

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The summary just given is disappointing in that it shows little uniformity in the results of the treatments. One point which is decisively brought out however, is the apparent quickening of growth caused by many of the treatments, beginning with the third period, growing longer again with the fourth period and remaining up until the eighth period, when the average number of days required for growth to begin suddenly declined and continued to decline through the ninth period.

The lack of uniformity of results in this table in part, may be attributed to the fact that the number of species treated was so small. A close scrutiny of the table, however, is interesting in that it shows, on the whole, that the tendency or, ability to grow, rises and falls during the course of the winter. This feature of the results is shown more clearly in the following table, which shows the average number of days required for growth to begin under each treatment, regardless of the dates on which treatments were given and in similar manner the percentages that grew are given:

TABLE XV.

Treatment.	Av. No. days for growth to begin	Percentage that grew.
Control	21.6	41.87
Etherised 24 hours	16.0	11.09
Etherised 24 + 24 hours	17.3	50.53
Etherised 48 hours	17.3	50.3
Etherised 48 + 48 hours	20.7	53.57
Dried 1 day	28.6	41.91
Dried 2 days	31.3	40.37
Dried 3 days	31.3	38.90
Dried 4 days	22.3	29.79
Dried 5 days	22.4	34.61
Dried 6 days	26.2	16.56
Dried 7 days	17.6	20.13
Dried 8 days	23.9	14.09
Dried 9 days	28.6	15.13
Dried 10 days	15.6	10.64

Two tests were made with dormant twigs to find if they would give off more moisture when etherized—that is, while exposed to the ether vapor—than when merely enclosed in a glass jar but not treated. During the course of the experiments the previous year it was noticed that there seemd to be more moisture in the etherized jars than in the controls.

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Two methods were employed in making the tests: In the one case hygrometers were placed in the jars and readings taken at different times covering a period of a few days; in the other case weighed quantities of calcium chloride were placed in the jars and afterwards reweighed to show the amount of moisture absorbed.

Hygrometer Test.—Two bundles of dormant twigs were placed in large glass jars and a hygrometer and a self-registering max. and min. thermometer so arranged in each that they could be easily read from the outside. In one jar was poured thirteen grams of ether (which was at the rate of forty grams per one hundred liters of space) and the other left untreated for comparison. Both jars were kept tightly closed during the eight days the experiment lasted. The experiment began early in the afternoon of January 24, 1907, and ended on February 1, following. The hygrometric reading showed fifty per cent of moisture in the jars before the twigs were put in. The temperature was 20° C. Following is an account of the readings in both jars:

TABLE XVI.

Etherized and both jars closed at 2 o'clock p. m., January 24, 1907.

OBSERVATIONS.	Humidity.		Temperature.	
	Control.	Etherised.	Control.	Etherised.
	Per cent.	Per cent.	Deg. C.	Deg. C.
Original Humidity and Temperature ...	50	50	20	20
After 3 hours (Jan. 24, 5 p. m.)	80	80	21.1	21.1
After 17¾ hours (Jan. 25, 7:45 a. m.) .	85	87	17.8	17.8
After 29¾ hours (Jan. 26, 7:45 p. m.)	82.5	85	21.1	21.1
After 34 hours (Jan. 26, 12 m.)	81	82.5	22.7	22.7
After 37 hours (Jan. 26, 3 p. m.)	81	82.5	22.7	22.7
After 9 days (Feb. 1)	85	85	18.9	18.9

Calcium Chloride Test.—Bundles of dormant twigs as nearly alike as possible were placed in two large glass jars. In each were also a thermometer, hygrometer, and 100 grams of calcium chloride. One jar was treated with 10½ grams of ether, this being at the rate of 40 grams per 100 liters of space. The experiment lasted three days—from February 9th to 12th inclusive. The same self-registering maximum and minimum thermometers employed before were again used. The results are shown in the following table:

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TABLE XVII.

Etherized and both jars closed at 12 o'clock noon February 9, 1907.

OBSERVATIONS.	Humidity.		Temperature.	
	Control.	Etherised.	Control.	Etherised.
	Per cent.	Per cent.	Deg. C.	Deg. C.
Original Humidity and Temperature ...	50	50	20.5	20.5
After 4½ hours (Feb. 9, 4:30 p. m.) .	74	79	20.5	21.1
After 27 hours (Feb. 10, 2:30 p. m.) ..	80	82.5	16.7	17.8
After 46½ hours (Feb. 11, 10 a. m.) ..	75	82.5	20.5	21.1
After 57½ hours (Feb. 11, 9 p. m.) ...	75	80	17.8	18.8
	Grams.	Grams.		
Original weight of Calcium Chloride ...	100	100		
Weight after 3 days (Feb. 12)	125	135		
Increase in weight	25	35		

In Table XVI it is observed that the amount of moisture increased rapidly in both the etherized and the control jars during the first three hours after treatment—the increase in each case being 30 per cent. At the next observation, however, it is noticed that, while the moisture continued to increase, the etherized jar showed the highest percentages. This continued to be true up to the end of 37 hours, after which time both jars gradually declined until the difference in their reading was only one and one-half per cent. The temperature of the room fluctuated considerably.

The ether evidently caused a decided acceleration in the transpiration—the maximum activity occurring at the end of about 17 hours after treatment. It will further be noticed that at this observation the temperature reached its lowest point, a condition which no doubt helped to increase the humidity.

A final reading was taken on February 1st, more than a week afterward, at a time when the temperature was low, both jars showing the same amount of moisture—85 per cent. The cause of the decline in both jars was no doubt due to the fact that the vapor condensed on the glass and flowed to the bottom. After this, although the temperature was low at the last reading, the percentages of the etherized and control jars were the same, the water on the sides and bottoms of the vessels in both cases tending to equalize the humidity as measured by the hygrometers.

In previous temperatures, where the room temperature fluctuated but little, it was noticed that the vapor usually condensed on the sides of the jars in about 20 hours after treatment.

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In Table XVII are noted the results of the second attempt, to find whether ether causes dormant twigs to give off more moisture than similar twigs untreated. At the first observation, 4½ hours after treatment, the etherized jar contained five per cent more moisture than the control. The percentage of moisture continued to be higher in the etherized than in the control up to the end of 57½ hours, when the difference was again five per cent. The difference here was twice as great as in the previous trial.

Table XVII also shows the approximate total amount of humidity (in the form of both vapor and water) present in the jars, the calcium chloride having absorbed the moisture. The data showed the presence of 10 grams more water in the etherized jar than in the other. Reference to the table, however, shows that at the end of the experiment the atmosphere in the jars was still quite humid but the etherized one was several per cent higher than the control.

In these two tests an effort was made to find whether etherizing the twigs caused any effect—direct or indirect—on the temperature. In Table XVI the figures showed that the temperature remained the same in both jars throughout the experiment. In the next test, however, there was a difference in the temperatures of the two jars beginning with the first observation, after 4½ hours, and continuing up to the last, there being as much as 1.1 degrees C. more warmth in the etherized jar, than in the control. This is very surprising when it is remembered that ether, in vaporizing, is expected to lower the temperature, and that transpiration also is a cooling process. On the other hand it is well known that considerable heat may be generated by respiration. In this case, because readings were not taken frequently enough and owing to the fact that the methods were not very exact, it is difficult to say with any degree of certainty whether the amount of heat generated was really sufficient to overcome the process of cooling apparently indicated by the data. This is a special problem in connection with the main question of the nature of the rest period and the actual effects of anesthetics in arousing dormant or "resting" plants into growth. Additional experiments are planned to be carried out next winter and in the meantime the figures in Table XVII are submitted for what they are worth.

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RESEARCH BULLETIN NO. 2.

UNIVERSITY OF MISSOURI

COLLEGE OF AGRICULTURE

AGRICULTURAL EXPERIMENT STATION

A Study of the Cause of Wide Variation in Milk Production by Dairy Cows

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COLUMBIA, MISSOURI
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A STUDY OF THE CAUSE OF WIDE VARIATION IN MILK PRODUCTION BY DAIRY COWS.

C. H. ECKLES and O. E. REED.

It is a well understood fact that cows under all conditions vary widely in both quantity of milk and fat produced, and in the economy of this production. It is not uncommon, and in fact is usually found that in a herd of ordinary size certain animals will produce twice as much milk and butter fat, or even more, during the year as other animals of the same age, of the same breeding, and having the same feed and care.

The importance to the producer of dairy products of taking advantage of this wide variation in economy of production has been emphasized very strongly in recent years by experimental work and by investigations of actual conditions as they exist among the dairy cattle of the country. The exact cause of this wide difference in economy of production has not as yet been pointed out. The investigation here reported was taken up for the purpose of determining the real cause of this difference. This line of work was suggested by the striking difference in production of two cows in the College herd.

These cows are registered Jerseys and are a little more than half sisters, the sire being the same and the mothers distantly related. The better cow of the two is Pedro's Ramaposa 181160, designated in this report by her herd number, No. 27. The inferior cow is Pedro's Elf 197242, designated as No. 62. These cows were raised under practically the same conditions according to the usual plan followed in our herd. They were fed their mothers' milk for some two weeks and then changed to skim milk. With this skim milk was fed a small amount of corn meal with hay in the winter and grass in the summer. Table 1 gives the facts in detail regarding the age and production of these two cows through the first two milking periods.

TABLE I.

PRODUCTION OF NO. 27 AND NO. 62, FIRST TWO LACTATION PERIODS.

	No. 27.	No. 62.
Date of birth	Sept. 4, 1902.	May 11, 1903.
Age at first calving	29 mo.	18 mo.
Lbs. milk first lactation period	4552	878
Lbs. fat first lactation period	238.8	44.1
No. days in milk	337	131
Lbs. milk second lactation period	7174	3189
Lbs. fat second lactation period	377	114.8
No. days in milk	365	232

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Table 2 gives a summary to date of the milk and fat records of all the daughters of Minette's Pedro, the sire of these two cows, that have been in the herd.

TABLE II.

RECORDS OF THE DAUGHTERS OF MINETTE'S PEDRO.

	No. Lactation Periods.	Av. Lbs. Milk.	Av. Lbs. Fat.
Pedro's Ramaposa	3	6750	365.8
Pedro's Elf	3	2225	109.4
Pedro's Alphea Elf	5	6151	309.8
University May	3	4723	227.0
Columbia Huguita	5	6322	273.1
Pedro's Daisy Bate	2	3456	193.7
Missouri Daisie	1	4910	205.5
University Daisie	4	7746	405.6
University Stella	3	5336	273.7
University Elf	4	5053	247.3
University Belle	3	4960	223.8
Pedro's Grace Briggs	3	4909	287.9
Pedro's Matron	3	6582	355.9
Miss Missouri	3	6844	331.0
Pedro's Emily Harris	3	5271	238.5
Pedro's Estella	2	8807	462.1
Pedro's Alphea Ward	1	4728	267.0
Pedro's May Hubbard	4	4073	184.9
Pedro's Virginia Meredith ..	3	5776	320.6

It will be noted that here we have a typical illustration of the wide variations that occur in the productive capacity of dairy cattle, even where they are of almost identical breeding and where conditions under which they are raised and kept are the same. Taking the production of the first two years together No. 27 produced 2.8 pounds of milk and 3.9 pounds of fat for each pound produced by No. 62. These two cows are not the only extremes in dairy capacity among the daughters of Minette's Pedro, as will be noted from Table 2. Two others have made records for a year above the best made by No. 27, while one other at least is as inferior as No. 62.

While the second milking period of these two cows was in progress and the wide variations above noted were observed in the production of the two animals an investigation was planned for the purpose of determining accurately the real cause of this extreme difference in efficiency as dairy cows. It was believed that if the cause of the variation in production between these two cows could be determined accurately, it would largely solve the question as to the variations in productive capacity among dairy cows in general.

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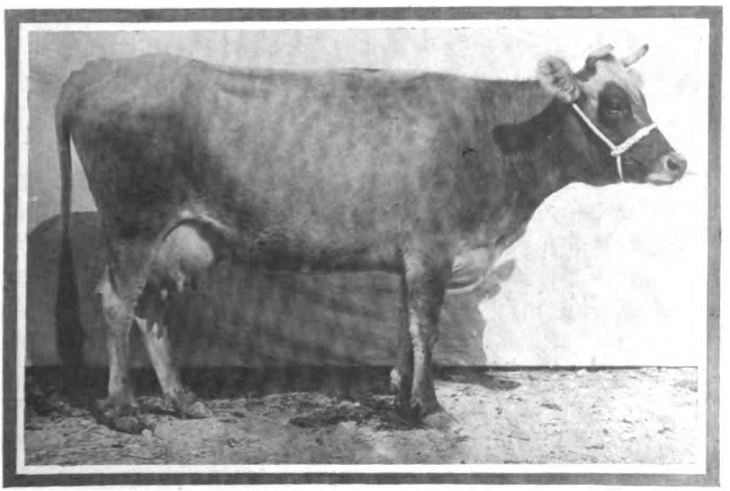
With this purpose in view the cows were bred so that the calves might be born as near as possible at the same time. No. 62 calved October 4, 1907, and No. 27, October 7.

Possible Causes of the Variation in Production.—In considering this subject with the object of preparing plans for an investigation the author concluded that the possible causes of variation must be among those given below. These statements are arranged in the reverse order of what was judged to be the probable importance:

A. Variation in the ration of maintenance.
B. Difference in the co-efficient of digestion of food by the two animals.
C. The production of body fat by the inferior producer from a portion of the ration given above maintenance.
D. Difference in the amount of food consumed and used in excess of the ration of maintenance.



NO. 27, PEDRO'S RAMAPOSA 181160.

Discussion of Possible Causes.—(A) It was not anticipated that there would be any marked difference found in the ration of maintenance, although it was considered possible so that it would have to be taken into account. To determine the ration of maintenance it would be necessary to keep the two animals for some time while farrow under maintenance conditions.

(B) The consensus of opinion among recognized authorities on animal nutrition seems to be that while the data regarding the co-efficient of digestibility with different animals is inadequate and conflicting, there is at present no reason for believing that there is any marked variation with individuals regarding the power of digestion.

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However. this possibility is one that has to be considered in such investigations as this and eliminated if of no importance. To determine this, digestion trials made while producing milk would be necessary.

(C) The possibility of the inferior cow using the excess feed for depositing fat on the body is suggested by practical observation which indicates that inferior milkers are liable to take on fat. This, in some cases, might be sufficient explanation of the variation in production by different dairy cows. In planning the experiment it seemed best to control this factor rather than attempt to measure it and with this object in view it was planned to maintain the cows at a uniform weight, and in this way eliminate this possibility. It is evident in considering this question that there is a limit to the deposition of fat on the body of the animal having such a tendency and that the consumption of food must necessarily be smaller at some time during the year with such an animal than with a heavy producer. If the cow fattens while producing milk she will need to accumulate little, if any, fat while dry before beginning her next milking period and for this reason will need a light ration while dry. On the other hand, the heavy milking cow may be rather thin at the end of the milking period and continue eating heavily while dry in order to accumulate the normal amount of fat on the body before the beginning of the next milking period.

(D) In analyzing the subject it was believed the main possibility for variation lay in the difference in the amount of food consumed and used by the cows above the amount required for maintenance. If one cow is born with the characteristic of producing large quantities of milk this will result in a correspondingly large demand for nutrients to replace that drawn from the body. This will result in the cow consuming large quantities of feed above maintenance. It is a common observation that heavy milk producing cows are heavy eaters. Another cow with less capacity and tendency to produce milk will not have as much removed from her body in the way of nutrients and, therefore, she will have a smaller appetite. In the case of an animal of this type it would be safe to assume that if fed *ad libitum* she will store fat on the body for a time until the tissues are well filled, then the appetite will drop until she will consume not more than is necessary to furnish maintenance and sufficient material for the milk she is producing. If it is assumed that the nutrients for maintenance will be the same for animals having wide difference in capacity for production, the economy of production, that is, the amount of feed required to produce a given quantity of milk or fat might vary immensely from this cause alone.

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Plan of Experiment.—The following statement gives the plan as made some months before the beginning of the investigation. It was carried out exactly as prepared.

"The first thing to be determined is the amount of food consumed by each animal. Both are to be given the same ration; that is, the grain mixture and the proportion between the amount of roughness and the grain is to to be the same for both. This proportion can be changed at any time when conditions seem to indicate that it is necessary but when it is changed for one it is to be changed for the other. The ration is to be adapted to the heavier producing cow. The grain ration shall consist of a mixture of at least three grains, preferably corn, bran, and oilmeal, and for roughness corn silage and alfalfa hay. A considerable quantity of each of these feeds, except the silage, is to be set aside for the exclusive use in this experiment in order to have as little change as possible in the composition of the feed. Each lot of feed used shall be sampled for chemical analysis. Both animals are to be fed sufficient food to maintain the body weight as near constant as possible. Both cows are to be given as large a ration as they will consume unless they begin using it for a gain in weight. It is probable that at least with No. 27 the weight will decrease for four or five weeks, regardless of how she is fed but this declining weight is to be checked as soon as possible and then kept at a uniform weight, or allowed to gain slowly until she returns to the original weight at the beginning of the milking period. Each animal is to be weighed at a stated time each day and a record of these weights kept. Accurate records are to be kept of the grain and roughness fed and of the amounts of each which may be refused. The refused feed of each animal is to be kept separate in a burlap sack and at intervals of ten days a sample is to be taken which represents the two mixed together. The dry matter in this sample is to be determined at once in the chemical laboratory and later a complete analysis is to be made. The same sample will be sufficient for both animals.

The two cows are to be placed in adjoining stalls and the same routine in general followed as is usual with the dairy herd. Care must be taken that the cows are not allowed access to any feed other than that in the regular ration. One man shall have complete charge of the two animals and do all the barn work connected with the experiment, if practical. Careful notes must be kept of any unusual conditions observed and general observations recorded regarding the condition of the animals, feeding characteristics, etc.

Sampling and Analyzing the Milk.—The milk produced by each cow is to be weighed in the usual manner and the weight recorded on the sheet in the barn. Pint glass bottles marked with the number

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of the cow and labeled morning and night are to be used for holding the samples. The milker is to mix the milk thoroughly with a dipper and fill the proper bottle about two-thirds full of the milk, placing a paper cap on the top of the bottle on which is marked the number of pounds of milk produced by the cow at that milking. This milk is to be brought to the Dairy Laboratory at each milking. The person having the sampling in charge takes a composite sample from this original sample by taking a definite number of cubic centimeters per pound of milk with a graduated pipette and placing it in a two-quart jar properly labeled. When the first lot of milk is placed in this jar, one cubic centimeter of formaldehyde is to be added and later at the end of the ten day period covered by the composite sample another one-half centimeter is to be added.

The composite samples made as described will cover ten days each, corresponding to the ten-day periods into which the experiment is to be divided. The samples, when complete, are to be analyzed by the chemist for fat, nitrogen, sugar and ash.

Digestion Experiment.—During the early part of the milking period, preferably about the third month, a digestion experiment is to be conducted with these two animals. Care must be taken that the ration is properly adjusted to preserve constant weight and that they have been getting the same ration for at least a month before this time in order that conditions may be entirely normal. About two weeks before this experiment begins the cows should be accustomed to being watered in the stable and to being kept in during the day in order that conditions may not be abnormal when the digestion trial is under way. The digestion trial will probably cover ten days time.

Maintenance Experiment.—Both cows are to be kept farrow and after both are dry, a maintenance experiment is to be conducted for at least 90 days using a ration of the same composition, or approximately the same, as fed while they were producing milk."

In most investigations with dairy cows, as, for example, feeding trials, the results are measured in three terms: i. e., feed consumed, milk and fat produced and gain or loss in live weight. It will be noted that the plans as drawn eliminated one of these factors making it possible to measure the results in two factors—feed consumed and milk and fat produced. In the beginning four possible causes of the variation between the two cows was given. All four of these are provided for by the plan.

(A) By a maintenance experiment at the end of the milking period with the cows farrow.
(B) By a digestion trial when the cows were at their best production.
(C) Eliminated by maintaining constant weight.

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(D) Uniform ration for the two and complete feed records and analyses of feed consumed and milk produced.

Summary of the Plan.—The following gives a summary of the plan as drawn and as carried out:

1. Complete record of amount and composition of feeds consumed.
2. Ration fed the two cows to be of the same composition at all times, the amount to be varied to suit the individual.
3. Cows to be kept at uniform weight.
4. Complete records made of milk produced and of its composition.
5. Cows to be kept farrow.
6. Digestion trial to be conducted when the cows are at their maximum production.
7. Cows to be kept on maintenance for at least three months at end of milking period to determine maintenance. Maintenance ration to be of same composition as that fed when producing milk to determine maintenance in terms of typical dairy ration.

Ration.—The ration selected for the two cows was alfalfa hay, corn silage and a grain mixture of which corn composed 4 parts, bran 2 parts, and oilmeal 1 part. The grain ration was made up in the same proportions throughout the entire year. The alfalfa hay used was of the grade known as "Choice." The hay was cut in an ordinary cutting box before being fed. The silage was of good quality, made from well matured corn in the usual manner.

Beginning with April 2nd green alfalfa was fed until the 24th of July when green corn was substituted and fed until the 15th of September as shown by the feed record in Table 5. During a portion of this time silage was also fed and a portion of the time green feed was fed alone. When feeding silage or green feed a composite sample was taken by placing a small sample of the feed fed each day in a glass container in which a small amount of chloroform was kept to prevent decomposition. The moisture was determined in the composite sample at the end of 10 days and a sample for chemical analysis made up by adding three or more of the air dry samples together.

Feeding and Management.—The two cows were kept in adjoining stalls in the barn during the night and a portion of the day when the weather conditions were unfavorable outside. They were turned outdoors in a lot each day where they had access to water which was warmed by a tank heater in cold weather. They had no possible opportunity to gain access to food except what was fed them in the barn. They were fed twice daily at the usual time for feeding the remainder of the herd, in the morning and evening. The grain was first fed and then the roughness. The hay was all fed in the evening after the grain was consumed while the silage or green feed was fed twice daily.

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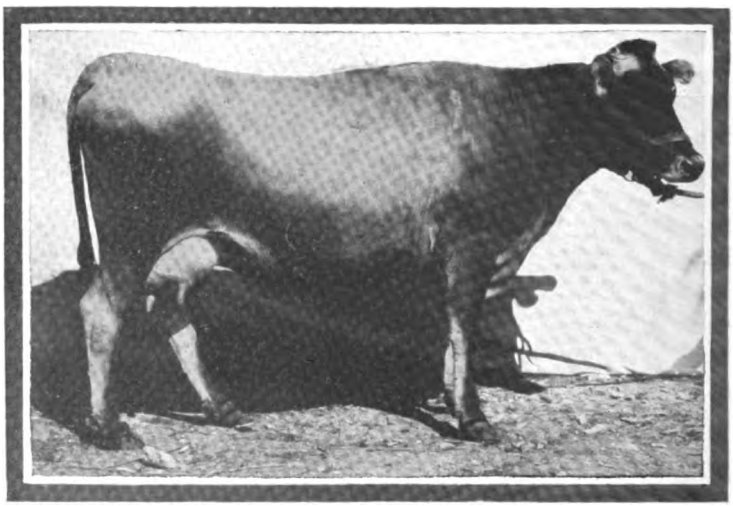
The same man milked both these cows in all cases. The animals were observed closely each day and notes made at frequent intervals regarding their condition. It was found unnecessary to make provision for weighing back feed as was anticipated when making the plan, since during the entire period of winter feeding there was scarcely 20 pounds of refused feed and this was mostly cobs from corn in the silage. The animals remained in exceptionally good health throughout the period covered by the experiment. Each animal was, on two occasions, given a dose consisting of from 1 to 1¼ pounds Epsom salts on account of slight indications of indigestion.

It was observed in general that No. 27 when producing the maximum milk yield, was practically to the limit of her capacity for handling food. It was judged from observation that during the months



NO. 62, PEDRO'S ELF 197242.

while producing the maximum amount of milk she would have consumed more grain but that she would not have consumed any more roughness. Her maximum capacity for food seemed to coincide closely with the amount necessary to maintain her at uniform weight. No. 62 consumed all her feed at all times and it was judged from observations that she would have taken a small amount additional had it been offered although she at no time showed lack of food. In observing the two animals at feeding it was very evident that No. 27 had much the stronger appetite. She ate her feed rapidly, swallowed the grain with much less chewing and always showed by her impatience to get her feed a much keener appetite than did No. 62. Both animals remained in splendid physical condition throughout the entire investigation with the slight exception noted.

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The digestion experiment was carried out as planned beginning on December 28, 1907 and continuing for 10 days. The daily ration was varied in quantity only when the live weight showed a gain or a loss. Weights were taken each morning after feeding and before watering. In studying the daily variation in weights it was found convenient to plot them at intervals of three or four days on sectional paper in order that the general trend might be better understood.

The average weights by 10 day periods are shown in Tables 4 and 5. It will be noted that No. 27 averaged 924 for the first period which consisted of only six days and then declined until the third period where the average weight was 846. This decline was foreseen in preparing the plan. It is impossible to feed a heavy milking cow a sufficient ration during the first month after calving to prevent a loss in weight. With No. 27 we assumed that if we replaced the weight lost the general result would be the same as it would have been had the weight been kept at a uniform point. With this object in view her weight was allowed to increase slightly during the greater part of the year. The intention was to close the year with the weight at practically the same point at which it was started. The table shows that during the last ten day period the average weight was 946 pounds, a gain of 22 pounds from the average weight in the first period. This gain, however, is very small in view of the fact that the period covered by the experiment is an entire year. As was expected, No. 62 did not decline in weight to any extent after calving. On account of the smaller production of milk it was found much easier to maintain her weight fairly constant than was the case with No. 27. The variations as shown by the average for to day periods are found in Table 5. While it was the intention to keep her at uniform weight, as a matter of fact there was a slight gain during the year since the average weight for the first 10 days was 888 pounds and for the last period of six days, 907. This gain, however, was practically the same for both animals and since it is so small it is not taken into account in our calculations. The usual difficulties were experienced in attempting to maintain the uniform weight. Whenever the ration is changed in character, as, for example, silage taken from the ration or added, the animals seem to change to a somewhat different plane due undoubtedly to a difference in the contents of the alimentary canal. Table 3 gives the daily variations in the weights of the two cows in two representative periods. This gives a fair idea of the ordinary variation from day to day.

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TABLE 3.

DAILY VARIATIONS IN WEIGHTS OF NO. 27 AND NO. 62.

(In Two Periods.)

Jan.	No. 27	No. 62	April	No. 27	No. 62
14	898	898	3	900	910
15	880	910	4	920	910
16	875	890	5	885	910
17	880	885	6	900	925
18	870	890	7	895	920
19	895	890	8	890	920
20	885	880	9	900	930
21	875	895	10	890	920
22	870	900	11	910	925
23	890	900	12	910	915
Average	882	894		900	919

No. 62 showed the same characteristics as in her two former milking periods and declined rapidly in milk production after four or five months. She was dried up on the 20th of August when she was producing about 3 pounds of milk a day, but the feed records cover the full 365 days. No. 27 continued to produce milk throughout the entire 365 days. At the close of her year she was producing approximately 14 pounds a day. Tables 4 and 5 give the average weights and a summary of the feed consumed by each of the two animals by 10 day periods. Tables 6 and 7 give a summary of the food constituents. It will be noted that the food constituents are not calculated digestible nutrients but the amount of the several constituents as found by chemical analysis of the food consumed.

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TABLE 4.

Cow No. 27.

SUMMARY OF FEED CONSUMED.

(Weights in Pounds.)

Period No.	Date.	Grain.	Alfalfa Hay.	Silage.	Green Feed.	Average Weight of Cow.
1	10-10—10-15	44	54	180	...	924
2	10-16—10-25	72	91	125	...	857
3	10-26—11- 4	75	90	265	...	846
4	11- 5—11-14	105	90	350	...	861
5	11-15—11-24	110	90	350	...	890
6	11-25—12- 4	110	90	350	...	879
7	12- 5—12-14	110	90	350	...	872
8	12-15—12-24	110	90	350	...	861
9	12-25— 1- 3	110	90	350	...	873
10	1- 4— 1-13	110	90	365	...	865
11	1-14— 1-23	110	88.5	370.5	...	882
12	1-24— 2- 2	110	90	326.7	...	865
13	2- 3— 2-12	110	83	338	...	875
14	2-13— 2-22	110	90	331	...	872
15	2-23— 3- 3	110	90	328	...	885
16	3- 4— 3-13	110	89	350	...	883
17	3-14— 3-23	110	90	325	...	897
18	3-24— 4- 2	101	90	300.5	...	888
19	4- 3— 4-12	100	90	320	...	900
20	4-13— 4-22	94	86	310	12	902
21	4-23— 5- 2	90	56	269	135	899
22	5- 3— 5-12	90	50	220	268	902
23	5-13— 5-22	90	79	191	314	914
24	5-23— 6- 1	90	56	233	300	924
25	6- 2— 6-11	83	50	219.8	260.8	925
26	6-12— 6-21	80	50	234.4	274.4	929
27	6-22— 7- 1	80	45.6	168.3	184.3	926
28	7- 2— 7-11	80	50	120.1	297.4	932
29	7-12— 7-21	80	76	...	304.1	899
30	7-22— 7-31	80	80	...	289.2	901
31	8- 1— 8-10	80	80	...	400	906
32	8-11— 8-20	80	80	...	400	928
33	8-21— 8-30	80	80	...	400	935.5
34	8-31— 9- 9	80	80	...	326	938
35	9-10— 9-19	80	80	160	160	938.5
36	9-20— 9-29	80	80	315.1	...	937.5
37	9-30—10- 9	80	80	312.2	...	956.5
Total		3424	2904.1	8777.9	4825.2	...

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TABLE 5.

Cow No. 62.

SUMMARY OF FEED CONSUMED.

(Weights in Pounds.)

Period No.	Date.	Grain.	Alfalfa Hay.	Silage.	Green Feed.	Average Weight of Cow.
1	10- 6—10-15	74	90	30	...	888
2	10-16—10-25	62	85	49.5	...	875
3	10-26—11- 4	58	78	204	...	882
4	11- 5—11-14	62	54.5	206	...	882
5	11-15—11-24	70	60	220	...	884
6	11-24—12- 4	70	60	220	...	882
7	12- 5—12-14	70	60	220	...	880
8	12-15—12-24	70	60	220	...	894
9	12-25— 1- 3	70	60	220	...	895
10	1- 4— 1-13	70	60	230.5	...	886
11	1-14— 1-23	70	60	244.5	...	894
12	1-24— 2- 2	70	60	220	...	898
13	2- 3— 2-12	62.8	55.8	210.2	...	875
14	2-13— 2-22	65	53	207	...	872
15	2-23— 3- 3	63.5	51.4	200.2	...	912
16	3- 4— 3-13	60	49	190	...	906
17	3-14— 3-23	60	49	190	...	909
18	3-24— 4- 2	60	53.5	208	...	907
19	4- 3— 4-12	60	54	210	...	919
20	4-13— 4-22	54	47.6	177.4	12	913
21	4-23— 5- 2	50	20.9	155.2	84.9	910
22	5- 3— 5-12	50	28	125.1	148.4	910
23	5-13— 5-22	50	44	108.7	173.1	919
24	5-23— 6- 1	50	31.3	136.5	166	925
25	6- 2— 6-11	45	27.1	129.4	160.7	926
26	6-12— 6-21	40	25	123.7	145	923
27	6-22— 7- 1	40	25	124.6	117.5	931
28	7- 2— 7-11	33	21.1	55	185	929
29	7-12— 7-21	30	28.6	...	120.3	911
30	7-22— 7-31	30	30	...	117	897
31	8- 1— 8-10	34	34	...	170	884
32	8-11— 8-20	37	37	...	185	905.7
33	8-21— 8-30	9	37	...	185	913
34	8-31— 9- 9	30	30	...	126	906
35	9-10— 9-19	30	30	60	60	904.5
36	9-20— 9-29	30	30	120	...	911
37	9-30—10- 5	18	18	72	...	907
Total for year		1907.3	1697.8	5087.5	2101.9	

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TABLE 6.

Cow No. 27.

SUMMARY OF FOOD CONSTITUENTS.

(Weights in Pounds.)

No. Period	Date.	Dry Matter.	Protein.	Nitrogen-free Extract.	Crude Fibre.	Ether Extract.	Ash.
1	10-10—10-15	126.523	17.245	69.385	27.575	4.229	8.168
2	10-16—10-25	149.223	11.710	25.373	39.544	5.759	11.710
3	10-26—11- 4	204.644	26.065	106.764	51.545	6.678	13.626
4	11- 5—11-14	248.282	31.599	134.431	57.860	8.636	15.831
5	11-15—11-24	252.639	32.280	137.479	58.093	8.866	15.968
6	11-25—12- 4	252.639	32.280	137.479	58.093	8.866	15.968
7	12- 5—12-14	283.124	33.379	160.394	62.615	9.353	17.430
8	12-15—12-24	283.124	33.379	160.394	62.615	9.353	17.430
9	12-25— 1- 3	283.124	33.379	160.394	62.615	9.353	17.430
10	1- 4— 1-13	287.518	33.678	163.080	63.677	9.476	17.677
11	1-14— 1-23	287.716	33.598	163.467	63.522	9.499	17.702
12	1-24— 2- 2	276.299	32.914	156.298	60.965	9.160	17.008
13	2- 3— 2-12	282.256	34.809	165.014	48.782	16.547	17.105
14	2-13— 2-22	286.838	35.604	165.629	50.509	17.373	17.720
15	2-23— 3- 3	285.902	35.536	165.045	50.324	17.319	17.675
16	3- 4— 3-13	291.817	35.893	168.927	51.404	17.683	17.909
17	3-14— 3-23	284.965	35.468	164.461	50.139	17.264	17.630
18	3-24— 4- 2	269.318	33.654	154.352	48.204	16.156	16.950
19	4- 3— 4-12	262.344	35.031	149.696	46.276	13.762	17.308
20	4-13— 4-22	252.992	34.501	143.238	45.948	12.948	16.424

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TABLE 6. (*Continued.*)

Cow No. 27.

SUMMARY OF FOOD CONSTITUENTS.

(Weights in Pounds.)

Period. No.	Date.	Dry Matter.	Protein.	Nitrogen-free Extract.	Crude Fibre.	Ether Extract.	Ash.
21	4-23— 5- 2	240.351	34.080	134.971	44.094	11.784	16.204
22	5- 3— 5-12	256.396	38.634	137.835	50.312	11.677	18.622
23	5-13— 5-22	258.869	38.703	136.201	53.136	11.831	18.999
24	5-23— 6- 1	254.803	36.183	136.259	52.593	11.245	16.527
25	6- 2— 6-11	242.252	34.911	128.726	50.468	10.291	17.856
26	6-12— 6-21	257.019	37.352	134.609	55.280	10.362	19.464
27	6-22— 7- 1	212.265	31.275	113.140	43.048	9.341	15.460
28	7- 2— 7-11	269.573	37.641	144.548	59.445	10.789	17.148
29	7-12— 7-21	256.619	45.092	120.620	59.257	9.875	21.773
30	7-22— 7-31	215.284	33.424	112.324	45.098	8.736	15.694
31	8- 1— 8-10	233.104	31.789	127.712	48.162	9.960	15.472
32	8-11— 8-20	249.204	32.061	133.500	53.216	9.918	17.706
33	8-21— 8-30	262.704	32.969	141.680	58.296	10.204	19.544
34	8-31— 9- 9	278.151	35.705	153.087	59.492	11.567	18.341
35	9-10— 9-19	246.427	31.633	134.697	54.462	9.941	15.685
36	9-20— 9-29	239.553	30.762	131.251	52.583	9.613	15.334
37	9-30—10- 9	238.773	30.699	130.806	52.390	9.586	15.283
Total .		9362.636	1224.985	6333.097	1951.590	404.995	521.793

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TABLE 7.

Cow No. 62.

SUMMARY OF FOOD CONSTITUENTS.

(Weights in Pounds.)

Period.	Date.	Dry Matter.	Protein.	Nitrogen-free Extract.	Crude Fibre.	Ether Extract.	Ash.
1	10- 6—10-15	155.886	24.296	86.182	30.356	5.228	9.856
2	10-16—10-25	145.018	21.854	70.340	32.940	4.675	9.489
3	10-26—11- 4	165.975	21.223	85.426	42.861	5.285	11.215
4	11- 5—11-14	147.755	18.820	79.785	34.620	5.116	9.444
5	11-15—11-24	162.777	20.842	88.150	37.780	5.666	10.360
6	11-24—12- 4	162.777	20.842	88.150	37.780	5.666	10.360
7	12- 5—12-14	181.989	21.533	102.553	40.644	5.972	11.266
8	12-15—12-24	181.989	21.533	102.553	40.644	5.972	11.266
9	12-25— 1- 3	181.989	21.533	102.553	40.644	5.972	11.266
10	1- 4— 1-13	185.014	21.742	104.399	41.388	6.069	11.457
11	1-14— 1-23	189.115	22.021	106.960	42.379	6.174	11.710
12	1-24— 2- 2	181.989	21.533	102.553	40.644	5.972	11.266
13	2- 3— 2-12	174.493	21.445	100.890	31.239	10.060	10.860
14	2-13— 2-22	172.884	21.271	100.019	30.499	10.466	10.626
15	2-23— 3- 3	167.908	20.682	97.167	29.567	10.176	10.312
16	3- 4— 3-13	159.322	19.622	92.147	28.106	9.645	9.800
17	3-14— 3-23	159.322	19.622	92.147	28.106	9.645	9.800
18	3-24— 4- 2	169.229	20.665	97.446	30.463	10.134	10.519
19	4- 3— 4-12	162.867	21.452	92.928	28.853	8.444	10.689
20	4-13— 4-22	144.712	19.763	82.005	26.218	7.394	9.400

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TABLE 7. (*Continued.*)

Cow No. 62.

SUMMARY OF FOOD CONSTITUENTS.

(Weights in Pounds.)

Period.	Date.	Dry Matter.	Protein.	Nitrogen-free Extract.	Crude Fibre.	Ether Extract.	Ash.
21	4-23— 5- 2	127.946	17.955	73.114	22.590	6.323	8.457
22	5- 3— 5-12	143.819	21.543	77.108	28.126	6.522	10.401
23	5-13— 5-22	143.972	21.554	75.972	29.303	6.627	10.518
24	5-23— 6- 1	143.708	20.256	76.966	29.738	6.310	10.442
25	6- 2— 6-11	138.502	19.922	73.196	29.316	5.736	10.333
26	6-12— 6-21	132.395	19.184	69.210	28.659	5.268	10.077
27	6-22— 7- 1	126.806	18.085	67.086	26.964	5.177	9.467
28	7- 2— 7-11	118.049	16.372	63.123	26.406	4.614	7.536
29	7-12— 7-21	96.660	17.367	46.179	22.944	3.745	8.425
30	7-22— 7-31	82.560	12.757	43.041	17.400	3.329	6.029
31	8- 1— 8-10	99.069	13.511	54.278	20.469	4.234	6.575
32	8-11— 8-20	116.029	14.832	61.805	24.666	4.586	8.214
33	8-21— 8-30	96.239	11.294	48.224	25.619	3.056	8.046
34	8-31— 9- 9	104.606	13.415	57.557	22.385	4.348	6.897
35	9-10— 9-19	92.410	11.862	50.511	20.423	3.728	5.882
36	9-20— 9-29	90.383	11.580	49.534	19.855	3.628	5.786
37	9-30—10- 5	54.230	6.948	29.721	11.914	2.174	3.472
Total .		5927.632	690.731	2890.880	1102.529	223.121	349.319

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A summary of the total feed and food constituents consumed by the two animals is shown in the following statements:

FEED CONSUMED DURING THE YEAR.

Summary of Tables 4 and 5.

(Weights in Pounds.)

No.	Grain.	Alfalfa Hay.	Green Feed.	Silage.
27	3424	2904	4325	8778
62	1907	1696	2102	5066

FOOD CONSTITUENTS CONSUMED DURING THE YEAR.

Summary of Tables 6 and 7.

(Weights in Pounds.)

No.	Dry Matter.	Protein.	N. fr. Ex.	Crude Fibre.	Ether Extract	Ash.
27	9362.6	1224.9	6338.1	1951.6	406.0	521.8
62	5927.6	690.7	2890.9	1102.5	223.1	349.3

RATIO OF FEED CONSUMED BY NO. 27 TO THAT USED BY NO. 62.

	No. 62.	No. 27
Grain	1	1.79
Hay..	1	1.71
Silage	1	1.72

It will be noted from the statement above that the plan to keep the ration of the same composition for each was carried out closely. At certain times the ration of one was changed slightly for some reason and this accounts for the slight variation in ratio of the grain, hay and silage. In general No. 27 consumed 1.75 pounds of feed for 1 pound consumed by No. 62.

Tables 8 and 9 give the yield of milk by 10 day periods for the two cows and the analyses of the composite samples representing these periods. Tables 10 and 11 give the yield of milk constituents by periods and the totals for the complete lactation periods.

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TABLE 8.

YIELD OF MILK AND AVERAGE COMPOSITION.

No. 27.

Period.	Lbs. Milk.	% Fat.	% Nitrogen.	Protein. N x 6.38.	Sugar.	Ash.
1	181.3	4.20	.52	3.32	5.00	.745
2	292.6	6.20	.52	3.32	4.70	.744
3	310.4	5.60	.52	3.32	5.30	.745
4	325.5	5.25	.55	3.51	5.00	.723
5	314.3	5.30	.56	3.57	3.55	.736
6	289.2	5.10	.58	3.70	3.76	.730
7	287.6	5.60	.59	3.76	4.40	.764
8	283.0	5.40	.58	3.70	4.86	.698
9	272.3	5.10	.61	3.89	5.03	.762
10	274.9	5.60	.62	3.96	5.30	.691
11	266.7	5.10	.64	4.08	4.30	.721
12	240.5	5.60	.62	3.96	4.96	.677
13	243.1	5.80	.64	4.08	4.83	.750
14	234.6	5.75	.64	4.08	4.95	.727
15	245.6	5.60	.66	4.21	5.08	.776
16	243.9	5.75	.65	4.15	4.66	.812
17	244.2	5.70	.65	4.15	3.90	.775
18	235.0	5.70	.67	4.27	5.30	.771
19	231.6	5.60	.68	4.34	4.96	.789
20	242.3	5.60	.66	4.21	4.60	.787
21	243.9	5.50	.67	4.27	4.90	.737
22	236.6	5.55	.68	4.34	4.50	.758
23	218.4	5.50	.66	4.21	4.50	.754
24	222.2	5.70	.65	4.15	3.60	.852
25	204.0	5.90	.62	3.96	4.70	.841
26	191.1	5.70	.64	4.08	4.50	.767
27	173.8	5.70	.61	3.89	4.67	.711
28	194.3	5.50	.63	4.34	4.69	.660
29	189.3	5.40	.63	4.02	4.80	.704
30	182.8	5.40	.63	4.02	4.08	.655
31	183.5	5.00	.65	4.15	4.50	.672
32	182.2	5.30	.67	4.27	4.93	.646
33	173.7	5.70	.62	3.96	4.58	.597
34	184.9	5.60	.67	4.27	4.93	.660
35	161.6	5.60	.71	4.53	3.79	.705
36	162.6	5.80	.70	4.47	4.33	.706
37	149.4	6.00	.69	4.40	4.35	.689

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TABLE 9.

YIELD OF MILK AND AVERAGE COMPOSITION.

(By Periods.)

No. 62.

Period.	Lbs. Milk.	% Fat.	% Nitrogen.	Protein N x 6.38.	Sugar.	Ash.
1	115.9	5.14	.63	4.02	5.06	.763
2	139.6	5.80	.63	4.02	4.85	.764
3	147.3	5.20	.63	4.02	5.30	.761
4	156.4	5.00	.62	3.96	3.42	.770
5	150.2	5.30	.62	3.96	6.48	.765
6	137.5	5.10	.63	4.02	3.41	.750
7	135.2	5.60	.63	4.02	3.93	.761
8	138.4	5.55	.63	4.02	4.61	.702
9	134.9	5.10	.65	4.15	4.55	.768
10	123.2	5.20	.62	3.96	5.00	.700
11	119.4	5.60	.62	3.96	4.23	.750
12	114.5	5.40	.63	4.02	4.43	.757
13	112.0	5.55	.62	3.96	3.93	.754
14	104.7	5.45	.65	4.15	3.52	.759
15	104.8	5.65	.66	4.21	6.18	.700
16	103.8	5.30	.65	4.15	4.48	.712
17	96.8	5.20	.63	4.02	3.88	.735
18	96.5	5.50	.64	4.08	4.85	.778
19	87.9	5.10	.66	4.21	4.51	.785
20	90.9	5.40	.60	3.83	4.30	.720
21	84.4	4.95	.58	3.70	5.00	.657
22	77.6	5.45	.64	4.08	4.70	.837
23	72.6	5.30	.61	3.89	4.40	.800
24	79.3	5.50	.60	3.83	3.50	.916
25	73.0	5.30	.62	3.96	4.50	.723
26	68.0	5.20	.64	4.08	4.20	.732
27	65.5	5.30	.63	4.02	4.40	.655
28	64.6	5.20	.60	3.83	4.61	.718
29	56.2	5.20	.59	3.76	4.58	.686
30	49.2	5.10	.58	3.70	4.55	.688
31	45.6	5.00	.60	3.83	4.50	.690
32	41.0	5.10	.60	3.83	4.25	.689

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TABLE 10.

YIELD OF MILK CONSTITUENTS BY PERIODS.

No. 27.

Period.	Date—10 days, Ending.	Lbs. Milk.	Lbs. Fat.	Lbs. Nitrogen.	Lbs. Protein.	Lbs. Sugar.	Lbs. Ash.
	1907						
1*	October 15	181.3	7.615	.943	6.016	9.065	1.351
2	October 25	292.6	18.141	1.522	9.710	13.752	2.177
3	November 4	310.4	17.382	1.614	10.297	16.451	2.312
4	November 14	325.5	17.069	1.790	11.420	16.275	2.353
5	November 24	314.3	16.658	1.760	11.229	11.158	2.313
6	December 4	289.2	14.749	1.677	10.699	10.874	2.111
7	December 14	287.6	16.106	1.697	10.827	12.654	2.197
8	December 24	283.0	15.282	1.641	10.470	13.754	1.975
	1908						
9	January 3	272.3	13.887	1.661	10.597	13.697	2.075
10	January 13	274.9	15.394	1.704	10.872	14.570	1.900
11	January 23	266.7	13.602	1.707	10.891	11.468	1.923
12	February 2	240.5	13.468	1.491	9.513	11.977	1.628
13	February 12	243.1	14.100	1.556	9.927	11.742	1.823
14	February 22	234.6	13.490	1.501	9.576	10.205	1.706
15	March 3	245.6	13.754	1.621	10.342	12.476	1.906
16	March 13	248.9	14.024	1.585	10.112	11.366	1.980

* 6 days.

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17	March 28	244.2	13.919	1.587	10.125	9.524	1.393
18	April 2	235.0	13.395	1.575	10.049	12.455	1.312
19	April 12	231.6	12.970	1.575	10.049	11.487	1.327
20	April 22	242.3	13.569	1.590	10.202	11.146	1.307
21	May 2	243.9	13.415	1.634	10.425	11.951	1.798
22	May 12	236.6	13.131	1.609	10.265	10.647	1.793
23	May 22	218.4	12.012	1.441	9.194	9.828	1.647
24	June 1	222.2	12.665	1.444	9.213	7.999	1.893
25	June 11	204.0	12.086	1.265	8.071	9.588	1.716
26	June 21	191.1	10.893	1.228	7.803	8.600	1.466
27	July 1	173.8	9.907	1.060	6.763	8.116	1.236
28	July 11	194.3	10.687	1.321	8.428	9.113	1.282
29	July 21	189.3	10.222	1.193	7.611	9.086	1.333
30	July 31	182.8	9.871	1.152	7.350	7.458	1.197
31	August 10	183.5	9.175	1.193	7.611	8.258	1.233
32	August 20	182.2	9.657	1.221	7.790	8.962	1.177
33	August 30	173.7	9.901	1.077	6.871	7.955	1.037
34	September 9	184.9	10.354	1.239	7.905	9.116	1.220
35	September 19	161.6	9.050	1.147	7.318	6.125	1.139
36	September 29	162.6	9.431	1.138	7.260	7.041	1.148
37	October 9	149.4	8.964	1.081	6.574	6.499	1.029
	Total	8522.9	469.965	53.194	339.875	392.458	62.508

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TABLE 11.

YIELD OF MILK CONSTITUENTS BY PERIODS.

No. 62.

Period.	Ten Days, Ending.	Lbs. Milk.	Lbs. Fat.	Lbs. Nitrogen.	Lbs. Protein.	Lbs. Sugar.	Lbs. Ash.
	1907						
1	October 15	115.9	5.957	.731	4.664	5.888	.884
2	October 25	139.6	8.097	.879	5.608	6.771	1.067
3	November 4	147.3	7.660	.928	5.921	7.807	1.121
4	November 14	156.4	7.820	.970	6.189	5.349	1.204
5	November 24	150.2	7.961	.931	5.840	9.733	1.149
6	December 4	137.5	7.013	.866	5.525	4.689	1.031
7	December 14	135.2	7.571	.852	5.436	5.313	1.029
8	December 24	138.4	7.681	.872	5.563	6.380	.972
	1908						
9	January 3	134.9	6.880	.877	5.595	6.138	1.036
10	January 13	123.2	6.406	.764	4.874	6.160	.862
11	January 23	119.4	6.686	.740	4.721	5.051	.896
12	February 2	114.5	6.183	.721	4.600	5.072	.867
13	February 12	112.0	6.216	.694	4.428	4.402	.844
14	February 22	104.7	5.706	.681	4.345	3.685	.795
15	March 3	104.8	5.554	.692	4.405	6.477	.784
16	March 13	103.8	5.501	.675	4.307	4.650	.789
17	March 23	98.8	5.138	.622	3.968	3.833	.726

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18	April 2	96.5	5.308	.618	3.943	4.680	.751
19	April 12	87.9	4.483	.580	3.700	3.964	.690
20	April 22	90.9	4.000	.545	3.477	3.900	.654
21	May 2	84.4	4.178	.490	3.126	4.220	.555
22	May 12	77.6	4.229	.497	3.171	3.647	.650
23	May 22	72.6	3.848	.443	2.826	3.194	.581
24	June 1	79.3	4.362	.476	3.087	2.776	.726
25	June 11	73.0	3.869	.453	2.890	3.285	.528
26	June 21	68.0	3.536	.435	2.775	2.856	.498
27	July 1	65.5	3.472	.413	2.635	2.882	.429
28	July 11	64.6	3.359	.388	2.475	2.978	.461
29	July 21	56.2	2.922	.382	2.118	2.574	.386
30	July 31	49.2	2.509	.285	1.818	2.289	.338
31	August 10	45.6	2.280	.274	1.748	2.052	.315
32	August 20	41.0	2.091	.246	1.569	1.743	.282
	Total	3188.9	169.385	19.970	127.297	144.397	23.900

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The statements below give a summary of the yield and average composition of the milk, the yield of milk constituents for the two cows, and the ratio between the yields of milk and milk constituents.

AVERAGE COMPOSITION OF MILK.

Summary of Tables 8 and 9.

Cow No.	Yield of Milk.	% Fat	% Nitrogen.	% Protein.	% Sugar.	% Ash.
27	8522.9	5.51	.624	3.98	4.60	.733
62	3188.9	5.31	.626	3.99	4.52	.746

COMPARISON OF YIELD OF MILK CONSTITUENTS.

Summary of Tables 10 and 11.

No.	Fat.	Nitrogen.	Protein.	Sugar.	Ash.
27	469.9	53.19	339.3	392.4	62.5
62	169.3	19.97	127.2	144.3	23.8

RATIO OF MILK AND MILK CONSTITUENTS PRODUCED BY THE TWO COWS.

Production of No. 62 represented by 1.

Milk.	Total Solids.	Fat.	Protein.	Sugar.	Ash.
1:2.67	1:2.72	1:2.77	1:2.66	1:2.71	1:2.62

It will be noted that No. 27 produced 2.67 pounds of milk and 2.77 pounds of fat for each pound yielded by No. 62.

In the beginning four possible causes of the variations in production were given under the letters A, B, C, D. It is now the purpose to discuss that data presented giving the feed consumed by the two animals during the year, and the production of milk and feed by each, with the view of showing its relation to these four possible sources of variation.

Maintenance Experiment.—It is not the purpose to give the full details regarding the maintenance experiment at this time. This data, however, will be presented in detail in a later publication. Only a summary and the totals are given which is necessary for making clear the relation of the results found by the maintenance test to the problem under consideration.

The maintenance experiment was carried out as originally planned with each of the two animals while dry and farrow. With No. 62 the

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maintenance period begun August 31, 1907 and continued 180 days, ending February 26. The feed records given for the lactation period for No. 62 in Tables 4 and 6 cover the entire year of 365 days beginning October 6, 1907. The time included from August 31, 1908 to October 5, therefore, overlaps and in addition to being counted as part of the year including the lactation period, is also a part of the maintenance period. This is possible since No. 62 was dry after August 20th while No. 27 was milked for the full year. The maintenance period for No. 27 included 160 days beginning October 30th and continuing until April 7th.

As stated in the plan of the experiment in carrying out this maintenance trial the general plan was to use the same feeds as were fed during the milking period and in practically the same proportions with the purpose in view of determining in terms of the ration fed while the cows were producing milk, how much of this ration was required for maintaining the animal. It is appreciated by the author that such a ration is not an economical maintenance ration and that one consisting of roughness alone and containing a much smaller amount of protein than was fed should be selected if the aim be to determine the most economical ration for maintenance. Such has been the aim of others who have carried out what little investigation there has been reported on the maintenance of dairy cattle. The object in view, however, in this case was not to select the most economical ration or determine the proper proportion of constituents for maintenance, but to find what part of the normal ration fed while the cows were in milk was needed by the animal for maintenance. The grain ration fed during the period of maintenance consisted of corn 4 parts, bran 2 parts and oilmeal 1 part which was exactly the same proportion used throughout the milking periods of the two animals. The same kind and quality of hay and silage was also fed as was used during the milking periods. In all cases the two cows had exactly the same ration with the exception of the necessary variation in the amount used. The ratio between the grain mixture, hay and silage was 1 :1 :4 which was about the average proportion fed while the cows were in milk. The cows were kept under the same conditions as described for the period when in milk and the two animals received the same treatment in every way. They were weighed each morning after being fed and before having access to water. The usual difficulties were experienced in maintaining the weights at a uniform point. On the whole, however, the results were as satisfactory as can be expected judging from the work of other investigators along this line. Both animals made a slight gain in weight during the maintenance period. In No. 27 this amounted to 21 pounds in 160 days, and in No. 62, 18 pounds in 180 days.

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TABLE 12.

SUMMARY OF FEED REQUIRED FOR MAINTENANCE.

	No. 27.		No. 62.	
Dates included	Oct. 30, 1908-April 7, 1909.		August 31, 1908-Feb. 26, 1909.	
No. days	160		180	
Average weight 1st 10 days.	881		906	
Average weight last 10 days.	902		922	
	Total.	Per Day.	Total.	Per Day.
Lbs. grain fed	525.9	3.29	526.4	2.92
Lbs. hay fed	527.9	3.30	526.4	2.92
Lbs. silage fed	2111.6	13.20	2116.6	11.76

Table 12 gives the data in regard to maintenance trial including the amount of hay, grain and silage used by each animal and the amount of each per day. The question was raised in the beginning regarding the possibility of variation in the amount required for maintenance. An examination of the data presented above shows that on the average No. 27 required a slightly larger amount of feed than did No. 62. It will be observed that the weight of the two animals is quite close and for this reason as well as for the fact that it interferes with the comparison from a practical standpoint the results are not calculated on a 1000 pound basis. On the average No. 27 consumed 3.27 pounds of grain per day for maintenance; No. 62 consumed 2.92 pounds and each had hay and silage in the same proportions. Below is given a comparison of maintenance requirements for an entire year. In calculating these figures it is assumed that the average food requirements found during the maintenance trial is the average for the entire year.

COMPARISON OF MAINTENANCE REQUIREMENT FOR ONE YEAR.

(Weights in pounds.)

	Grain.	Alfalfa Hay.	Silage.
No. 27	1200.8	1204.5	4818.0
No. 62	1065.8	1065.8	4292.4
Difference	135.0	138.7	525.6

It will be noted that on this basis it would require 135 pounds more grain, 138 pounds more alfalfa hay, and 525 pounds more silage to maintain No. 27 for a year than is required for No. 62. The data shows that undoubtedly there is a small difference in the maintenance require-

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ments of these two animals. It is evident that this difference does not account in any way for the wide variation in the production of the two animals. In fact, No. 27, the larger producer, has the higher maintenance requirement. The only conclusion that can be drawn from this data is that in this case the factor of variation in maintenance requirement is so small that it may be ignored in attempting to explain the difference in the economy of production.

Digestion Trial.—Under B in the discussion of the possible causes of variation in production a possible difference in the coefficient of digestion by the two animals was given. A digestion trial covering 10 days time was carried out, under the direction of Dr. P. F. Trowbridge of the Department of Agricultural Chemistry, as called for by the plan. This digestion trial began December 27, 1907. It is not the intention to give complete data regarding this digestion trial at this time. A summary only will be given so far as it relates to the question under investigation and full details will be published later with other data of similar character.

At the time of carrying out the digestion trial the animals were close to their maximum production of milk. The digestion trial was carried out in the usual manner. All the feed to be fed during the ten days was weighed out in advance by weighing the ration for each day by itself into a closed bucket. At the same time samples were taken for chemical analysis. All the solid and liquid excretion of the animals was collected by attendants and subjected to chemical analysis. Below is given a statement of the ration fed daily to each animal during this digestion trial and the average yield of milk and fat.

DAILY RATION AND MILK YIELD.

10 Day Digestion Trial.

(Weights in Pounds.)

	No. 27	No. 62
Alfalfa Hay	9	6
Grain	11	7
Silage	85	22
Average milk yield	26.8	18.3
Average fat yield	1.37	.68

All the data necessary for studying the relation of the digestion trial to the problems under consideration is given in Tables No. 13 and 14. These tables give a summary of the feed constituents consumed by each animal, the amount of each excreted, and the per cent digested.

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TABLE 13.

SUMMARY OF RESULTS—10-DAY DIGESTION TRIAL.

No. 27.

(Weights in Grams.)

	Consumed.	Excreted in Dung.	% Digested.
Protein	15094.44	6225.06	58.75
Fat	4228.80	1397.43	66.95
Crude Fibre	28090.08	12972.22	53.82
Nitrogen-free Extract	65982.21	19775.25	70.03
Total	113395.53	40369.96	64.39

TABLE 14.

SUMMARY OF RESULTS—10-DAY DIGESTION TRIAL.

No. 62.

(Weights in Grams.)

	Consumed.	Excreted in Dung.	% Digested.
Protein	90566.48	3771.25	60.58
Fat	2686.82	1079.39	59.82
Crude Fibre	17782.97	8198.14	53.89
Nitrogen-free Extract	41922.09	12143.83	71.04
Total	71958.36	25192.61	64.99

It will be noted by studying these figures that there is some variation in the per cent of the different food constituents digested by the two animals but in no case is the variation of any great extent. Taking the average digestion coefficient, as the per cent digested of the total constituents consumed, it will be observed that the results are remarkably close, No. 27 showing 64.39 per cent digested and No. 62, 64.99. It is evident from the above results that the wide variation in the production of these two animals cannot be attributed in any way to a difference in the power to digest food. The variation is less than would have been anticipated with any two animals that might have been selected regardless of dairy qualities.

(D) In the discussion of the possible sources of variation it was assumed that the main possibility lay in a difference in the amount of food consumed and used in excess of the ration of maintenance. It has been shown by Tables 4 and 5 that there was considerable difference

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in the amount of feed consumed during the year by the two cows and that No. 27 consumed 1.75 pounds of feed for every pound consumed by No. 62. A summary of the amount of feed consumed and the food constituents for the two cows is given on page 123

In Tables 15 and 16 is shown what part of the ration given the two cows was available for milk production. This table is made up by taking the first row of figures, the totals of feed consumed from Tables 4 and 5, and the second row, the estimated maintenance for the year, calculated as previously shown (P. 26), from the maintenance period of 160 days with No. 27 and 180 days with No. 62.

TABLE 15.

SHOWING PORTION OF RATION AVAILABLE FOR MILK PRODUCTION.

No. 27.

(Weights in Pounds.)

	Grain.	Hay.	Silage.	Green Feed.
Consumed during year in milk	3424.0	2904.0	8778.0	4325.0
Maintenance for year	1200.8	1204.5	4818.0	.....
Available for milk production.	2223.2	1699.5	3960.0	4325.0

TABLE 16.

SHOWING PORTION OF RATION AVAILABLE FOR MILK PRODUCTION

No. 62.

(Weights in Pounds.)

	Grain.	Hay.	Silage.	Green Feed.
Consumed during year in milk	1907.0	1698.0	5068.0	2102.0
Maintenance for year	1065.8	1065.8	4292.4	.....
Available for milk production.	841.2	632.2	795.6	2102.0

Attention will at once be drawn to the large proportion of the ration available for milk production with No. 27 as compared with that for No. 62. Since the two rations are made up in almost exactly the same proportions between grain, hay and silage, a direct comparison can be made between the amount available for milk production for the two cows by comparing the grain.

For every pound of grain available for milk production in the ration of No. 62 there was 2.64 pounds in the ration of No. 27.

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The ratio between the milk produced by the two cows as already shown was 1:2.67 and for the fat 1:2.77 which coincides remarkably close to the ratio between the feed actually used for milk production. The data then shows that after the ration of maintenance is deducted, No. 62 produces milk and fat as economically as No. 27. What feed is used for milk production by the inferior cow, No. 62, is used to as good advantage as is the case with the heavy producer. The difference in economy of production arises from the fact that the former requires the greater part of what she can digest to supply maintenance while with the heavy producer a large amount is used after maintenance is provided for. This comparison is illustrated below by expressing the portions of the ration used for maintenance and for milk production in per cent.

	Used for Maintenance.	Used for Milk Production.
No. 27	35.0 per cent	65.0 per cent
No. 62	55.8 per cent	44.2 per cent

Additional Data from Cows No. 4 and No. 63.—Fortunately we are able at this point to use data secured in carrying on another line of investigation, which was under way at the same time as the one heretofore described. It has already been stated that after deducting the ration of maintenance, No. 62 produced milk as economically as did No. 27 that produced more than twice as much. The object in introducing this additional data is to determine whether or not the same is true regarding other cows than the two used in the investigation and to compare the results from No. 62 and No. 27 with these other cows. The two cows that supply this additional data are No. 4 and No. 63.

These two cows are registered Jerseys and half sisters of No. 27 and No. 62. While they were used in another investigation they were fed practically the same as were the two animals which furnished the data heretofore given. No. 4 and No. 63 calved at almost the same time and were fed on the same ration as No. 27 and No. 62 with the exception that the proportion between the grain and hay was not kept entirely uniform. They were fed from the same mixture of grain at all times as was used for feeding No. 27 and No. 62, and all were fed from the same hay and silage. Furthermore, they were kept farrow during the year and were kept at uniform weight in exactly the same way as was done with No. 27 and No. 62. At the end of twelve months they were both dried up and were then kept on maintenance on the identical ration used for No. 27 and No. 62. With No. 4 the maintenance period covered 150 days and with No. 63, 120 days. Further and complete details regarding the feeding of these animals during the

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year they were producing milk and during the period of maintenance will be published later. At this time only the summaries are given which are necessary in order to study the question under consideration.

Table 17 gives a summary of the feed consumed by these two animals during the year.

TABLE 17.

SUMMARY OF FEED FOR YEAR.

No. 4 and No. 63.

No.	Grain.	Alfalfa Hay.	Silage.	Green Feed.
4	3035.3	3376.0	7084.3	2490.1
63	2968.0	3298.0	8046.7	2501.3

TABLE 18.

COMPOSITION AND YIELD OF MILK AND MILK CONSTITUENTS.

No. 4 and No. 63.

No. Cow.	Days in Milk.	Yield Milk.	Fat.		Protein.		Sugar.		Ash.	
			%	Yield.	%	Yield.	%	Yield.	%	Yield.
4	365	6773.6	5.50	372.9	4.12	278.3	4.28	290.2	.77	51.4
63	365	6033.9	6.09	367.9	4.37	263.8	4.21	254.3	.72	44.9

The proportion between the grain, hay and silage is much the same as was fed No. 27 and No. 62. Table 18 gives a summary of the yield of milk, its composition, and the total yield of milk constituents for the two animals. It will be observed that the two animals produced very near the same amount of milk but the milk of No. 63 contained on the average .59 per cent more fat. These two animals ranked between No. 27 and No. 62 in yield of both milk and fat. Tables 19 and 20 give a summary of the food consumed on maintenance by the two animals.

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TABLE 19.

SUMMARY OF FOOD CONSUMED ON MAINTENANCE BY NO. 4.

	Grain.	Alfalfa Hay.	Silage.	Average Weight.
150 days	513.9	514	2066.4	792
Average per day	3.43	3.43	13.78	...

TABLE 20.

SUMMARY OF FOOD CONSUMED ON MAINTENANCE BY NO. 63.

	Grain.	Alfalfa Hay.	Silage.	Average Weight.
120 days	353.4	353.4	1425.6	888
Average per day	2.95	2.95	11.88	...

These figures show that there is a difference in maintenance requirements in this case as was found with the other two animals, No. 4 requiring 3.43 pounds grain on the average to 2.95 pounds required by No. 63, the other parts of the ration being in the proportions. At the same time No. 63 weighed almost 100 pounds more. If we were to calculate the maintenance on a basis of 1000 pounds live weight, this variation would be still more marked. Tables 21 and 22 give the amount of feed available for milk production during the year the two cows were in milk. This is found as described for Tables 15 and 16 by subtracting the maintenance required for the entire year, calculated at the same rate as actually determined for short periods, from the total feed consumed during the year when milk is being produced.

TABLE 21.

FEED AVAILABLE FOR MILK PRODUCTION DURING 1 YEAR IN MILK.

No. 4.

	Grain.	Alfalfa Hay.	Silage.	Green Feed.
Feed consumed 1 year in milk	3035.3	3376.0	7064.3	2490.1
Maintenance for year	1251.9	1251.9	5029.7	.....
Available for milk production	1783.4	2124.1	2004.6	2490.1

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TABLE 22.

FEED AVAILABLE FOR MILK PRODUCTION DURING I YEAR IN MILK.

No. 63.

	Grain.	Alfalfa Hay.	Silage.	Green Feed.
Feed consumed 1 year in milk	2968.0	3296.0	8046.7	2501.3
Maintenance for year	1076.7	1076.7	4336.0	.....
Available for milk production	1891.3	1221.3	3710.7	2501.3

The ration consumed by No. 63 was somewhat different in proportions from the others and in order to compare the relative amount of hay, grain, silage, and green feed consumed in the rations of the four cows the following summary is made:

RATIO BETWEEN CONSTITUENTS OF THE RATION WITH THE FOUR COWS.

	Grain.	Alfalfa Hay.	Silage.	Green Feed.
No. 62	1	.89	2.67	1.10
No. 27	1	.85	2.56	1.26
No. 4	1	1.11	2.32	.82
No. 63	1	1.11	2.71	.84

The above shows that No. 4 and No. 63 had more hay and less green feed in proportion to the grain than was used by No. 27 and No. 62. However, the ration is not so far different in its composition. For this reason the statement below is prepared showing the ratio for the four cows between the available grain in the ration and the fat and milk produced, representing the figure for No. 62, by unity which is in each case the smallest.

COMPARISON OF FOOD AVAILABLE FOR MILK PRODUCTION ON BASIS OF GRAIN ALONE.

	No. 62.	No. 27.	No. 4.	No. 63.
Ratio grain available	1	2.64	2.12	2.25
Ratio fat produced	1	2.77	2.20	2.17
Ratio milk produced	1	2.67	2.12	1.89

The close agreement between the ratio of available food consumed and the fat in the milk produced has already been pointed out for No. 27 and No. 62. It will be seen from the statement above

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that with No. 4 it is correspondingly close while with No. 63 there is more variation. However, No. 63, as already shown, was given a ration varying considerably in the proportions between the grain and hay from that given the other animals, and furthermore her milk was considerably richer in fat than was the case with that produced by the other cows. These facts, are sufficient to account for the ratio being wider between the grain of this cow and No. 62 than is the ratio between the milk and fat.

Since these rations varied in composition as shown and the milk varied in composition as well, the following calculations are introduced, where by expressing both the value of the feeds in the ration, and the value of the solids in the milk in calories, it is possible to reduce both to a basis that admits of a fair comparison. In making these calculations the "production value" as given by Dr. H. P. Armsby, Bulletin 71 (Revised Edition), Pennsylvania Experiment Station, was used. Table 23 gives the value in calories for the average daily ration consumed by the four cows while in milk. Table 24 gives the value in calories of the average daily ration for the four cows while on maintenance. Table 25 gives the value of the milk solids computed in calories. In preparing this table the following values for the milk solids were used.*

Milk protein	Calories per gram	5.86
Butter Fat	Calories per gram	9.23
Milk Sugar	Calories per gram	3.95

The figures for the total yield of the several milk constituents will be found in Tables 10 and 11 for No. 27 and No. 62, and in Table 18 for No. 4 and No. 63.

*Hammarsten, Physiological Chemistry, p. 625.

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TABLE 23.

PRODUCTION VALUE IN CALORIES.

(Average Daily Ration in Milk.)

Nos. 27, 62, 4, 63.

	Calories Per Lb.	No. 27.		No. 62.		No. 4.		No. 63.	
		Lbs.	Total Calories.	Lbs.	Total Calories.	Lbs.	Total Calories.	Lbs.	Total Calories.
Corn	888.5	5.4	4797.9	3	2665.5	4.8	4264.8	4.7	4176.0
Bran	482.3	2.7	1302.2	1.5	723.5	2.4	1157.5	2.3	1109.3
Oilmeal	789.3	1.3	1026.1	.8	631.4	1.2	947.2	1.2	947.2
Alfalfa Hay ..	344.1	8.0	2752.8	4.7	1617.3	9.3	3200.1	9.0	3097.0
Silage	142.6	24.1	3436.7	13.9	1982.1	19.3	2752.2	22.1	3151.5
Green Alfalfa	106.1	6.6	713.5	3.5	378.4	3.6	389.2	3.6	389.2
Green Corn ...	110.2	5.3	584.1	2.2	242.4	3.3	363.7	3.2	352.6
Total			14,613		8,240.		13,074.		13,222.

TABLE 24.

PRODUCTION VALUE IN CALORIES.

(Average Daily Ration on Maintenance.)

Nos. 27, 62, 4, 63.

	Calories per Lb.	No. 27.		No. 62.		No. 4.		No. 63.	
		Lbs. Fed.	Total Calories.	Lbs. Fed.	Total Calories.	Lbs. Fed.	Total Calories.	Lbs. Fed.	Total Calories.
Corn	888.5	1.9	1688.2	1.7	1410.5	2.0	1777.0	1.7	1510.5
Bran	482.3	.9	434.1	.8	385.8	1.0	482.3	.8	385.8
Oilmeal	789.3	.5	394.7	.4	315.7	.5	394.7	.4	315.7
Alfalfa Hay	344.1	3.3	1135.5	2.9	997.9	3.4	1169.9	2.9	997.9
Silage	142.6	13.2	1882.3	11.8	1682.7	13.8	1967.9	11.9	1696.9
Total			5,534.		4,792.		5,791.		4,906.8

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TABLE 25.

VALUE OF MILK IN CALORIES.

(Weights in Pounds.)

	Value per Lb. in Calories.	No. 27.		No. 62.		No. 4.		No. 68.	
		Amount Produced.	Total Calories.	Amount Produced.	Total Calories.	Amount Produced.	Total Calories.	Amount Produced.	Total Calories.
Fat.	4190.4	469.9	1969069.0	169.3	709434.7	372.9	1562600.2	367.9	1541648.2
Protein	2660.0	339.3	90253.8	127.2	33835.2	278.3	74027.8	263.8	70170.8
Sugar	1793.3	892.4	703690.9	144.3	258773.2	290.2	520415.7	254.3	456086.2
Total .. .			2,763,014.		1,002,043.		2,157,044.		2,067,855.

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Table 26 gives a summary of Tables 23 and 24 and in addition shows the total calories per day available for milk production by each cow after the maintenance requirement is supplied. The ratio is then given between the available calories in the daily ration of the four animals while producing milk, and the ratio between the milk solids expressed in calories counting the production of No. 62 as unity in both cases.

TABLE 26.

SUMMARY OF CALORIES IN DAILY RATION, IN MILK AND ON MAINTENANCE; AND RATIO BETWEEN AVAILABLE CALORIES IN FEED AND IN MILK SOLIDS.

	No. 62.	No. 27.	No. 4.	No. 63.
Total Calories in average daily ration in milk	8241	14614	13075	13223
Total Calories in average daily ration on maintenance	4793	5535	5792	4907
Total Calories per day available for milk production	3448	9079	7283	8316
Ratio of available Calories, No. 62 counted as unity..	1	2.63	2.11	2.41
Ratio of Calories in milk produced, No. 62 counted as unity	1	2.75	2.14	2.06

It will be seen from Table 26 that the ratio between the value of the available feed in calories and between the milk solids expressed in calories is close in every case. This shows that after the maintenance requirement is supplied one used food as economically as another for milk production. What was found to be true as between No. 27 and No. 62 by comparing their rations directly by pounds fed, is found to be true as well for the four cows when the feed and milk product is expressed in calories.

Here we have four animals kept under identical conditions except a slight variation in the proportion between the grain and hay, all kept at uniform weight during the milking period and all kept farrow. We find that while there is some small difference in the maintenance requirements of the four animals, that after we subtract the maintenance from the total consumed that the ratio of their production is practically that of the available food. It seems to the writer that the evidence furnished by these four animals is so consistent that it is entirely safe to draw a general conclusion that as between these

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dairy cows at least and probably between others the difference in the economy of production is dependent upon the amount of food which they can consume and use above the ration of maintenance.

Results of Feeding No. 62 to the Limit of Her Appetite.—In studying the results of the experiment reported with the two cows one question that will naturally rise is this: What would have been the result had No. 62 been fed all the feed she would consume, in place of her ration being regulated by her live weight? It was concluded from observation that she would have taken somewhat more feed than she received during that part of the milking period when producing the maximum quantity, but she had at no time appeared hungry, and it was evident that she was almost to the limit of her capacity for food. Practical experience would indicate that she would not have produced more than a small additional amount of milk no matter how she was fed. At the same time the question remained unanswered as to what would have resulted had she been given all the feed she would have consumed.

The maintenance test already reported ended February 26, 1909. She was bred shortly after this and calved November 30, 1909. She was then put on a ration practically the same as given two years before while the data heretofore presented was being gathered. She was at first fed what would be considered a normal ration to support the amount of milk produced. In this milking period for the first few weeks she produced from 4 to 5 pounds more milk per day than ever before. This, however, could not be attributed in any way to a difference in the feed since she was fed practically the same ration as given before. It is not unusual for such variations to occur from one year to another in the amount of milk produced by the same animal due to conditions which cannot be determined.

Beginning with December 22nd the ration fed was gradually increased above the normal requirements for a cow producing that quantity of milk. By January 12 the ration was raised to 11 pounds grain per day and by the end of January to 13 pounds per day. As the grain ration was increased the consumption of hay and silage was decreased as will be seen from the data given in Table 27. As the grain was increased she refused the silage first and for that reason silage was dropped from the ration during the last period, February 10 to 19. From December 22 to February 19 she was fed all the feed it was possible to get her to consume. The result shows that her capacity for using food was but very little above the ration fed during the experimental milking period as reported in Table 5. It will be noted from Table 27 that there was no increase in the amount of milk as the feed was increased. More milk, in fact, was produced when she received

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8.4 pounds grain a day than was produced when she received 14.8 pounds grain per day. This proves that the small production of milk by this cow in the experimental period did not result from an insufficient ration. During the 70 days covered by the data presented in Table 27 the extra nutrients consumed above that necessary to provide for the milk was evidently used in depositing fat on the body as there was a gain in weight of 25 pounds in 70 days.

TABLE 27.

RECORD OF FEED CONSUMED AND MILK PRODUCED.

No. 62.

Period.	Date.	Lbs. Grain.	Lbs. Alfalfa Hay.	Lbs. Silage.	Value Daily Ration in Calories.	Total Lbs. Milk.	Average % Fat.	Average Weight.
2	12-12—12-21	59	78.5	150.0	9307	185.6		877
3	12-22—12-31	84	103.4	72.5	10961	190.7	5.4	875
4	1- 1— 1-10	93	91.0	106.5	11729	193.8	5.0	893
5	1-11— 1-20	112	87.0	74.3	12546	203.4	4.7	892
6	1-21— 1-30	130	79.4	75.3	13666	192.5	4.6	889
7	1-31— 2- 9	145	108.7	59.8	15581	189.0	4.6	902
8	2-10— 2-19	148.2	111.0		15059	187.2	3.6	902

In addition to its bearing upon the problem under consideration, the results have an important bearing on the problem of feeding. They show with this animal, what is unquestionably a general rule; that when a sufficient amount of feed is given to support milk production to the full capacity of an animal, that additional feed will not bring greater results. If the animal has not the stimulation, whatever it may be, necessary to produce milk it cannot be brought about by an increase in the ration.

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SUMMARY AND CONCLUSIONS.

During the first two lactation periods the better of the two Jersey cows in this investigation produced 2.8 pounds of milk and 3.9 pounds of fat for each pound produced by her half-sister. The object of this investigation was to determine the cause of this wide variation with these two cows, anticipating that an explanation as between these two cows would explain the cause of variation with dairy cows in general.

In the third lactation period the two cows calved three days apart and were kept under the following conditions:

The two were fed a ration of exactly the same composition.

The quantity was so regulated as to maintain a uniform weight.

Both cows were kept farrow.

Complete records were kept, and analysis made, of feed consumed and milk produced for the entire lactation period.

A ten-day digestion trial was carried out while the two cows were in milk.

A maintenance trial was conducted at the end of the milking period.

During the year of the investigation No. 27, the better cow, produced 8522 pounds of milk and 469.9 pounds of fat; No. 62 produced 3188 pounds of milk and 169.3 pounds of fat. No. 27 produced 2.67 pounds milk, and 2.77 pounds fat for each pound produced by No. 62.

No. 27 consumed 1.75 pounds of feed for each pound used by No. 62.

The maintenance trial showed only a slight difference in cost of maintenance, the higher requirement being with No. 27.

The digestion trial showed practically identical results. The co-efficient of digestion for No. 27 was 64.39 per cent and for No. 62, 64.99 per cent.

The real cause of the difference in production was found to be in the amount of feed consumed above maintenance.

No. 27 used 35 per cent of the total food consumed, and No. 62, 55.8 per cent for maintenance.

After deducting the maintenance requirement one cow produced milk as economically as the other.

Data is presented of two other cows kept for an entire lactation period under identical conditions as those above described.

The amount of food required for maintenance during the year is estimated for each cow individually from a trial covering a period of from 120 to 180 days.

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The feed consumed during the lactation year minus the estimated maintenance is the amount considered available for milk production.

The ratio between the food available for milk production and the milk produced is practically the same with each of the four cows.

The available feed consumed and the milk solids produced are also calculated in calories in order to reduce all to a common basis.

The ratio between the calories available for milk production with the four cows agrees closely with the ratio between the calories in the milk solids produced by the same animals.

This shows that with the four cows, as for the two made the special object of this investigation; the cause of the difference in the amount of milk produced is the amount of food they are able to consume and use above that required for maintenance.

The main difference between profitable and unprofitable dairy cows is not to be found in the coefficient of digestion, or in the amount of food required for maintenance.

A superior dairy cow is simply one with a large capacity for using food above the maintenance requirement and one that uses this available food for milk production.

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RESEARCH BULLETIN NO. 3

UNIVERSITY OF MISSOURI

COLLEGE OF AGRICULTURE

AGRICULTURAL EXPERIMENT STATION

Soils of The Ozark Region

A Preliminary Report on the General Character of the Soils and the Agriculture of the Missouri Ozarks

BY C. F. MARBUT, PROFESSOR OF GEOLOGY,
UNIVERSITY OF MISSOURI

COLUMBIA, MISSOURI
June, 1910

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SOILS OF THE OZARK REGION.

BY C. F. MARBUT.

INTRODUCTION.

This report is an attempt to determine the character, distribution, and natural fertility of the soils of the Ozark region as well as to suggest, in the light of the results of this study, the most profitable way of handling them. It is an attempt to direct attention to the proper channels and to find the best means of making the region yield a larger income. The results so far attained cannot be considered absolutely final but it is confidently believed that data have been already obtained upon which suggestions of considerable value to the farmers of the region may be based.

It is not a detailed report and does not therefore deal with all of the types of soil existing in that large area of country. The conclusions drawn concerning the soils, and the systems of agriculture adapted to them, are general only. It is merely a preliminary report and will be followed by detailed study as rapidly as the circumstances will permit.

Soil investigation in the region has long been needed. A large part of the area has a soil of only moderate fertility and therefore a soil that will not stand abuse indefinitely. It does not smile upon the farmer who refuses to treat it well and to supply it with food. It does not lavish great wealth on the soil robber simply because it has not the great wealth to expend upon him. On the other hand it is of such a character that it will not refuse to reward to the fullest extent the hand that feeds it. Its response to good treatment is prompt and full. It returns to the farmer products of many times more value than the value of the food that he gives it.

The Ozark farmer, however, has not learned to treat his soil like he treats his horse. He is unable to hear the cry of the starved soil as he hears that of his live stock, though the value of the response of the soil to good treatment is probably greater than that of live stock. The latter can move about from place to place and often gain sustenance regardless of treatment by the owner, the former cannot do so.

The roughness of the country and the occurrence of mineral deposits of considerable value in parts of it have caused the people to turn a great deal of their attention to mining, neglecting their farms. The land has become poor in proportion to this neglect.

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Agriculture, including Animal Husbandry, is the only permanent industry to which the Ozark region as a whole is adapted. There is a great deal of the region that can never, or probably will never, be converted into tillable land, yet for the region as a whole agriculture will become its most important industry. That part of it not adapted to agriculture will be utilized for growing timber.

The first few pages of the report deal with the reasons for having a soil survey. This is followed by a short description of the geography and geology of the Missouri Ozark region with chief stress laid upon the character and distribution of the different beds of rock that appear on the surface in the different parts of the region. This is followed by a short general description of the soils as a whole, after which the soils are subdivided into groups and each group described in terms of the character of rock from which it has been derived, its distribution, topography, timber growth, area in cultivation, and physical and chemical characters of the soil. The description of the soil groups makes up the greater part of the report. The agricultural conditions of the region are described in general terms including a short history of its agricultural development. It is shown that for the central part of the region the average farmer probably receives less income now than he received forty years ago. This is due to the decrease in the size of the bottom land farms resulting from subdivision, and to the destruction of the native grass by the growth of brush and timber. This latter condition is the result which has arisen because of the fencing up of a small part of the upland and the consequent stopping of the annual prairie and woods fires. It may be due in a few cases, also, to over grazing.

The fruit industry is considered briefly and the conclusion is reached that it can never constitute the great fundamental industry of the region. That must be stock raising and dairying. The land must be reset in grass.

THE REASONS FOR A SOIL SURVEY.

Modern society recognizes the intimate relations existing between the prosperity and happiness of mankind and the resources of the earth's crust. These resources constitute the raw material from which man obtains the supplies to satisfy his many and varied requirements. It is upon these that the very existence of man depends, and upon their continued abundance and availability depends all future progress.

The duration of the life of the human individual is short. He depends upon the resources of the earth's crust, at most, but a few years. He is directly concerned with the present and the immediate future, and ordinarily thinks or cares little about the distant future

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of the world. Human society in some form is, on the other hand, perpetual. It neither begins nor ends with the life or death of an individual or group of individuals. It is as much concerned with the distant future as is the individual with the immediate future. It must or should plan for all time.

If the resources of the earth's crust were inexhaustible there would be no occasion whatever for spending any time, thought or energy upon them in any way. It has long since been demonstrated, however, that under the conditions produced by the occupation of earth by a dense population of human beings the resources are not inexhaustible. That although none of these resources can be absolutely annihilated, yet they are being rapidly placed by man in positions where they are not easily available.

It is incumbent upon society, therefore, in order to plan intelligently for the future, to determine the nature, amount and availability of these resources. Every government, as the personification of a particular portion of human society, should determine these things within the area of its jurisdiction.

It was not until the 19th century had been well advanced that man began to realize these things to such an extent that any action was taken. Such action at first related only to the so-called mineral resources—those that are secured by mining. It is now a well recognized duty of the government of every civilized nation to determine the nature, amount and availability of its mineral resources. Every civilized government has well organized departments devoted to this work and a great body of men have been specially trained for it. In the United States this work has been vigorously prosecuted for nearly half a century by a strong central organization of the national government and by many efficient state organizations. Up to the closing years of the 19th century very little interest was manifested, especially by the American people, in the greatest of all the earth's natural resources: the soil. Great sums of money were spent on the investigations of mineral deposits as well as upon the various subsidiary industries that depend upon the soil as a foundation, such as animal husbandry, plant breeding and fruit growing, etc. Yet practically nothing was done toward determining the source of the soils fertility, the reserve store of it in the soil, its rate of consumption or the geographic distribution of the various soils of the country. Volumes had been written on growing and feeding crops, on breeding and care of animals and even on the treatment and improvement of the soil before a clear idea had been gained of what the soil is, what constitutes its crop producing capacity, or what, in any particular case, is its store of fertility. Mineral maps, geological maps, timber maps, crop maps

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and live stock maps have long been familiar articles of use to intelligent men, yet soil maps are even yet almost a curiosity. The study of the soil is not a recognized function of geological or mineralogical surveys. The attention that this work has been receiving during the last few years is due to the work of the United States Department of Agriculture and to the Agricultural Experiment Stations of the various States. They have originated the work, and in many states the stations are engaged in a more or less systematic investigation of the soils of the state in which they are located, while the work of the Bureau of Soils of the National Department of Agriculture undertakes to extend such work over the whole country. The attitude of the Experiment Stations arose naturally as one of the conditions for the improvement of agriculture.

As long as mining was unsystematic and intermittent; as long as large areas of the earth were unoccupied and the surplus population had an easy outlet to new and unexplored country and until modern industrial conditions produced a great demand for the products of mines, no attention was paid to the total supply of the minerals of the earth. It was assumed that the earth contained an abundance and much to spare. As long, likewise, as agriculture was a rule of the thumb and as long as the farmer could get a new farm of virgin soil as fast as an old one was worn out there was little thought or attention paid to the soil. With the exhaustion of the free public land in the United States and the change to an intensive agriculture due to high-priced land and a denser population there came a demand for an accurate knowledge of the soil and of the means of improving it.

In a modern mining industry in which the speculative element has been eliminated one of the first things to be determined, if possible, is the amount of ore or other product that can be made available on the property. On this and the quality of the material will depend to a great extent the plan of operation of the industry. The property is thoroughly prospected, the size, shape and quality of the ore body determined and the future of the industry planned accordingly.

Mining, however, is a temporary industry. It can exist in any particular locality only so long as there is some valuable product to mine. Agriculture on the other hand is a permanent industry. It must exist as long as human society. It is now and has long been recognized by thinking men that the soil, the fundamental basis of agricultural industry, must be looked upon somewhat in the same way as a deposit of mineral. The plant food in the soil corresponds to the ore in a mineral deposit. The growing of crops is the farmer's method of mining the valuable material out of the soil. A part of the plant

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food consists of minerals, and like all other mineral deposits, they exist in the soil in a definite quantity. The soil in this sense is, therefore, a mineral deposit which is being mined out by the peculiar mining method of the farmer.

Since agriculture must be permanent, however, the great problem is to devise a method by which the soil may be maintained in a permanently fertile condition in the cheapest possible way. The growing of crops is, however, more than a mere method of mining the fertility from the soil. It is a method of mining and manufacturing in one act, extracting the fertility from the soil and at the same time manufacturing it into products of much greater value than that of the unmanufactured elements, the raw material. The maintenance of the fertility depends upon the return to the soil in a cheap form of the elements taken from it and used by the plant in the making of the various products which we call crops. The amount and kind of the materials to be put into the soil can be determined accurately only through an intimate knowledge of what the soil has to start with, what its facilities are for holding them and delivering them up at the proper time and in the proper condition for the plant as well as what is being taken out by the crop. A knowledge of both these things is necessary; one equally so with the other. The former knowledge can be gained only by a study of the soil in the field and such a study of the soil in the field and laboratory is the legitimate function of a Soil Survey.

The other elements of fertility in the soil in addition to the mineral elements are not to be neglected. All the elements and conditions of fertility are important as objects of study. They are the objects of research as well as the mineral elements. The final limit of agricultural permanence and production will probably, in the end, be determined by the mineral fertility since this cannot be produced in the soil, and the total available quantity is small. If the soil is robbed of it, it must be carted back and replaced from some outside source. One of the main functions of the field division of a soil survey, therefore, in addition to determining the location of the different soils, is to determine the physical character and mineral content of the soil.

If society is sufficiently interested in the minable mineral deposits of a country to be willing to tax itself for the purpose of determining their probable quantity and quality, to a much greater degree should it demand the investigation of the soils on which its very existence depends. The mining industry of any region is temporary and recognized as such while agriculture is as permanent as society itself.

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THE MISSOURI SOIL SURVEY.

The Missouri soil survey was organized by the Missouri Agricultural Experiment Station in the spring of 1905. Its work was grouped under two heads; (I) The soil survey proper, including the determination of the field character and relations of the soils as well as their study in the chemical and physical laboratory; (II) the experimental study of certain types of soils by means of crop growing under greatly varying conditions. The several parts of the work were placed under the direction of the professors of the Agricultural College most directly concerned. The survey proper was placed under the direction of the Professor of Geology and the Crop Experiment work under the direction of the Professor of Agronomy.

Three seasons of three months each have been spent in the field. In addition to a great deal of data concerning the crop experiments and the chemical characters of many samples of soil all of which will be discussed later, reconnoissance work has been carried over the greater part of southern Missouri. A general examination of the main soil groups of the Ozark region has been made. The soils have been broadly classified, samples have been collected and analyzed and the results are shown in the soil map accompanying this report. Each kind of soil is described as to origin, character, distribution and general fertility in the first part of this report. The general agricultural conditions obtaining in the region are discussed in the second part.

THE OZARK REGION.

Location.

The Ozark region as a whole is an oval shaped area lying chiefly in southern Missouri and northern Arkansas. It extends a short distance into Oklahoma on the west and into Illinois on the east. A little less than half of the total area lies within the state of Missouri.

Boundaries.

The boundaries, if run exactly, would be very irregular, extending outward along every stream flowing out of the region and inward along every ridge. There is no universally recognized criterion or body of criteria for determining its boundary. Everyone recognizes the rather rough region of south central Missouri with its stony red or gray soils as the Ozark region but the change from the rough country

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of the Ozark interior to the smoother country of the prairies to the north and west is gradual. The soils of the Ozark region are not all stony and some soils outside of the region have reddish and brownish and yellowish colors. The most striking and most persistent feature characterizing the Ozark region, especially in Missouri, and differentiating it from the surrounding areas, is the practically universal occurrence in it of limestone. Limestone is the Ozark rock, so far as Missouri is concerned, *par excellence*. A few thin sandstone beds occur in it but they merely give a local character to the relatively small area in which they occur. The Ozark region is the region, in Missouri, of massive limestones. Its boundary is the line or belt between the country where limestone is the predominant rock and that where shale or sandstone or clay predominates. The northern boundary, from St. Charles to Arrow Rock, lies along the northern part of the hilly belt bounding the Missouri river valley on the north. Thence southwestward for a long distance, the boundary is not a sharp line. There is a rather gradual change on this side from the smooth or rolling prairies with their broad flat-bottomed shallow valleys to the rougher Ozark region with its narrow and often deep valleys. This indefiniteness is especially characteristic of the stretch from Arrow Rock to the Osage river at Taberville. It is less sharp on the uplands than on the streams. In fact the place where the boundary crosses the streams is usually easily located as the place where the valleys become narrow and where limestone and flint rock appear in the creek and river cliffs. From Arrow Rock the line runs southward crossing Salt Fork in the neighborhood of Blackwater. It then follows up Lamine river and Big Muddy creek to the neighborhood of the bridge over which the Lexington Branch of the Missouri Pacific railway crosses the latter stream. Thence it swings eastward around the edge of the Sedalia prairie crossing Flat Creek east of Green Ridge. It runs thence eastward to the Meridian of Cole Camp, and then, in general, westward to Tebo Creek ten miles below Calhoun. Thence southward, with an eastward loop around the Sandy prairie west of Leesville, crossing Grand river at Brownington and the Osage at Taberville, running eastward or southward on the ridges and northward or westward in the valleys. South of this it crosses Horse Creek not far from the west line of Cedar county with a deflection to the eastward on both sides of it, running south through Stockton. From that place it runs in a nearly direct line to Golden City and Jasper. Beyond this, it follows approximately the valley of Spring river. The other boundaries, except that from St. Charles to the Mississippi river in the southern part of the city of St. Louis, and that from Cape

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Girardeau to the state line south of Doniphan in Ripley county, lie outside the state.

General Character.

Of the 70,000 square miles in the area of the state about 30,000 are included within the boundaries of the Ozark region. An area including as large a proportion of the state as this will be an important factor in the state's industries. Its rate of progress or its industrial condition will profoundly affect the whole state in this and other respects. The estimation in which it is held as a favorable or unfavorable region for human exploitation and habitation will determine to a considerable extent the standing of the state.

The estimation of the value of a region for agricultural purposes depends greatly upon a comparison with the country surrounding it. The prairies lying north and west of the Ozark region are noted for their fertility. Their topography and climate also are favorable, in the highest degree, to an intense and profitable agriculture. They possess all the favorable conditions to an extraordinary degree; to a greater degree probably than any other area of equal size in the world.

The Ozark region on the other hand has, as a rule, a rather uneven surface; it is covered with timber or brush necessitating considerable expense in preparing the land for the plow; its soil is usually stony, making cultivation rather difficult and costly, and while its soil is not sterile as a rule yet it is less fertile than that of the surrounding region. Its climate is essentially the same as that of the surrounding areas in corresponding latitudes. In comparison, therefore, the region has some disadvantages.

As long as large bodies of fertile prairie land lay open for settlement or could be bought cheap, men were unwilling to do the work necessary to clear the stony timbered lands of the Ozark region. This has not only retarded its settlement but has kept the capital necessary for rapid and effective development out of the country.

The region as a whole is rough. Its streams all flow in valleys which usually are rather deep, narrow and steep-sided. Its roughness is, however, not so great as is ascribed to it by the popular idea. The popular misconception concerning the region is due to two causes. One of them, and probably the chief one, is the name that was early applied to it and which has remained attached to it even in the face of a clearer knowledge of the facts. The expression "Ozark Mountains" seen in print and placed on the maps in the old geographies has produced an impression that will be long in disappearing. This applies chiefly to the public as a whole who have not seen the region.

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Another reason for the misapprehension is its contrast with the surrounding country and to a considerable extent a contrast that does not concern the shape of the surface directly. The effect produced by its cover of timber and the contrast between its timber and the surrounding prairies has had a great deal to do with the popular idea of its roughness.

The impression produced by the great abundance of stones occurring everywhere on the surface has had its influence in the same direction also. Considerable areas of it are smoother than much of the best parts of northern Missouri. On the other hand, large areas of the region are rougher than any part of northern Missouri. The rough regions lie in the neighborhood of the large streams while the smooth areas are along the watersheds.

The roughness of the region, however, is invariably of a broader, less intricate and less minute type than that of the northern part of the state. In both regions the roughness is due to the erosion of valleys in a nearly level surface. In the northern part of the state many of the hilly areas along the streams are so intricately eroded, so minutely cut up with the steep hollows and ravines that it is impossible to do anything with the land except convert it into pasture. In south Missouri, on the other hand, the minute drainage lines are less abundant; the slopes are therefore broader and smoother, and are capable of cultivation as well as conversion into pasture.

The soil and topography of the region are the direct result and its covering of timber an indirect result of the character of the rocks that lie under it. If the same kind of rocks lay under the Ozark region as those lying under western Missouri and eastern Kansas, its surface would be the same as in those areas. The boundaries of the region are determined by the extent of the rock which gives it its characteristics. In this respect the boundary is geological. It is geographic also since the geology (rock) determines the character of the soil and the shape of the surface. The Ozark region in Missouri is, as a whole, a limestone region. With the exception of the relatively small granite and porphyry area of the eastern part of the Ozarks and other small areas with sandstone and shale, limestone is universal. It is exposed in every river and creek bluff and encountered in every well and drill hole that penetrates the solid rock. There are several different kinds of limestone ranging from pure to impure, flinty to flint-free, crystalline to earthy. The different kinds are of widely different ages also and each kind produces a different kind of soil.

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Geology.

Age of the Rocks.

All the true Ozarkian rocks are of great age—all older than the age of the coal deposits of the world and most of them much older. The oldest of these rocks are as old as the oldest rocks found in any part of the United States. They are so old that it might be said that their origin dates back to a period before the beginning of legible geological history.

The youngest of the Ozarkian rocks belongs to the age immediately preceding the coal. So far as our purposes are concerned, however, the age of the rocks of the region is of much less importance than is their character. It is rock character which determines soil character.

The Kinds of Rocks.

The rocks from which the soils of the Ozark country have been made by nature's agents of disintegration belong to two of the great rock groups of the earth's crust, i. e., massive rocks and bedded rocks. The massive rocks have supplied only a very small area of the soils of the region, not more than one third of one per cent of the total area. Their effect therefore on the soil of the state is almost negligible. Although the area where they come within the reach of soil-forming agents is small this does not represent their total extent beneath the surface of Missouri. In fact they lie beneath it all but so far beneath that they are rarely reached in our deepest drill holes. They are the foundation on which the superstructure of the state has been built. The superstructure has been worn away in a few places in the granite region of the eastern Ozarks exposing the foundation to the disintegrating agencies.

On top of this foundation was laid a great thickness of rock beds built or laid one on top of another. These beds or layers are chiefly made up of limestones as stated above and where they have been worn off least their total thickness is more than two thousand feet.

These layers of rock were laid down as great sheets of material on the bottom of a sea that extended far beyond the existing limits of the Ozark region. The successive sheets were piled one on top of another and they all lay originally sensibly horizontal. The youngest layer, or the one laid down last, lay on top and the oldest on the bottom.

The successive layers included not only the limestone layers that make up the Ozark region as we know it at the present time, but

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a great thickness of layers of mud, sand, limestone and coal beds that were laid on top of the limestone. The total thickness of the whole series from the granite foundation below to the top of the sands, clays and coals of the coal measures was at least 3000 feet.

Structure.

After this series of layers had been spread out one on top of another on the bottom of a sea that included the whole of the Mississippi basin and probably much more, the whole region was raised above the sea. Where the Ozark region now lies these layers were not only raised above the sea with the rest but they were bowed upward into a low, broad, gently sloping ridge, standing higher therefore than the newly uplifted land of the surrounding country. These successive changes are graphically shown in plate I. The upper series of three sections represents the lay of the rock beds along the line A, B, C, D (see the small sketch map accompanying it) as they were before they were bowed upward. The middle series of three sections represents the same beds after the upbowing. The crooked black line drawn across these beds nearly horizontal is drawn approximately where the present surface of the region lies in this series of beds. The lower series of three sections is a more accurate representation of the surface as it is at the present time. It shows also the relation of these to the various soil belts crossed by the section.

The center or highest point, immediately after the upbowing, was not exactly in the geographical center of the Ozark region but was east of this, in the Iron county region. It was only in the Ozark region that these beds were bowed up. Elsewhere they retained the same attitude that they had from the first.

Erosion.

The upbowing in what is now the Ozark region exposed the top of the upbowed part to more vigorous wear by the elements than the lower country around it. The top layers were worn off in that part that had been raised up higher than the surrounding country. The layers of sand, mud, clay and coal from the upbowed region were worn away exposing the limestone layers beneath them but left the former layers still overlying the latter in the lower country around the region. The wearing not only took off the upper layers but took off all of the layers of the limestones from the central part or the part that was bowed up the highest, and much of them from most of the rest of the area. The top limestone layer was removed from the largest area and the lower ones from successively smaller areas. In a small area around the Iron Mountain country in southeastern Missouri all the limestone layers were worn off exposing the old foun-

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dation on which the whole series had been laid. What we now call the Ozark region in Missouri is the country where the limestone beds have been uncovered, including also the small area from which all the layers of limestone have been removed.

This wearing process finally produced a nearly level plain, which extended over the whole Ozark region and which has, since it was formed, been cut up into valleys and ridges by the existing streams. Small remnants of that old level surface still exist in many places in the Ozark region like the high smooth country around Springfield, Rolla, Lebanon, Salem, Versailles and Owensville. Most of it, however, has been cut to pieces with valleys.

In this levelling off no one bed was wholly removed from the whole area of its original extent. None of the beds were worn off outside of the Ozark region because, as stated before, they were and still are covered by later beds. It was only in the upbowed Ozark region that they were worn away and the greatest number of the beds were cut through immediately under the highest part of the upbow. The deepest bed touched or reached by the leveling work was removed from the smallest area and the highest or top bed was removed from the largest area. This youngest layer was worn away until it has become nothing more than a rim or ring belt running around the outer edge of the region. The next lower bed makes a belt inside this one running around the region and each successive lower bed forms a ring, each one becoming smaller the nearer the center we come until we reach the center, where even the oldest layer has been removed and the granite foundation has been exposed. If nothing more had been done than merely the laying down of the successive layers of material, we should of course be able to see only the top layer; the older layers being covered by the younger ones.

Description of the Various Rock Beds.

The layers of rock, as stated above, are not all exactly alike though the most abundant rock, by far, is limestone. The bottom rock, the foundation of the original pile of layers and the one now seen in the center of the region surrounded by rings of the younger layers, is made up of igneous rocks in which granite is an important factor. All of this foundation rock has very much the same composition as granite and differs from it only in texture. So far as the soil which is produced from it is concerned it may all be called granite.

The next layer is a sandstone. It is not thick and does not underlie a broad area. It was not formed as a complete continuous layer but only in patches so it is found only in patches around the granite area. It is known as the LaMotte sandstone. Above the

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LaMotte sandstone occurs a limestone known in different parts of the Ozark region as the St. Joseph, the Proctor and the Fredericktown limestone. It is several hundred feet thick, is a continuous layer and is found all around the granite and sandstone area, except where it is covered by the next higher layer. Its character varies somewhat, being a somewhat bluish shaly limestone in some places and a gray highly crystalline limestone in others. Its most important characteristic, however, is the absence from it of flint layers and nodules. A thin sandstone comes next in the series. It is known as the Gunter sandstone. It seems to have been formed in a small area. It is seen, therefore, only in a few places and is not exposed at any place over a wide area. It has had practically nothing to do with soil making in the region.

The next layer is a limestone, several hundred feet in thickness, and exposed over a wide area of country. It consists of massive beds of crystalline limestone with very little shale or shaly limestone except in a small area in southeastern Missouri. Being a thick bed, its different layers are not all alike, but their chief differences so far as their relation to the soil is concerned lie in the varying amount of flint which occurs in them. Taken as a whole this formation or layer has more flint than any other layer of the whole Ozark region. It occurs however, chiefly in the top part of the formation with a smaller quantity in the bottom part. The middle beds are almost entirely free from it and in some places the same is the case with the bottom beds. The flint may occur in thin layers of an inch or so in thickness, in nodular or roundish masses of any size up to 6 or 8 feet in diameter or in great massive or broken and recemented layers of any thickness up to 20 feet. It is known as the Gasconade limestone.

The next layer is a sandstone. It is the thickest and most widely distributed sandstone layer in the whole Ozark region. It underlies rather large areas in the central part of the region. It does not extend entirely around the central core of the region in a belt of uniform width. It is found chiefly on the western side of the central core. It is known as the Roubidoux sandstone. In the ascending series the next is the Jefferson City limestone. It is a thick one and has a wide distribution—the widest of any of the rock layers of the region. It consists of massive crystalline layers, alternating with fine grained layers, called "cotton rock," and shaly layers. As compared with the preceding limestone layer it has a rather small percentage of flint. What flint it has is scattered through the formation rather evenly from top to bottom. It occurs usually in small roundish nodules or in thin beds. Rarely does it occur in massive beds.

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Above this lies another bed of sandstone which curves around the northern and eastern side of the Ozark core, instead of the western and southwestern as in the case of the Roubidoux sandstone. It is known as the Crystal City or Pacific sandstone.

Above the Pacific sandstone lie a series of rather pure limestones free from flint which form a belt of fertile soil. They include the series of beds known in Missouri as the first Magnesian limestone and the Trenton limestone. They occur now chiefly in the eastern part of the region. Their area of occurrence is chiefly in Perry and Cape Girardeau counties.

Above this lies a thin layer of shale that is more or less continuous around the western, northern and eastern parts of the region but in a belt so narrow that it is unimportant except in a small area of southwestern Missouri. It is known as the Hannibal shale.

Finally the last layer, one that is continuous around the whole Missouri part of the region, west, north, and east, is a layer of limestone. It is about 250 feet thick but in southwestern Missouri it has a wide distribution. In the other parts of its curving area its belt is not so wide. It is made up chiefly of beds of massive crystalline pure limestone with a rather large amount of flint. The latter occurs in rather small nodular masses or in thin beds. It is known as the Mississippian limestone.

This is the top layer of the Ozark region proper and the outer ring, therefore, of this series of ever-widening rings of country.

In tabular form these rocks, beginning with the highest, lie as follows:

11. Limestone, flinty, "Mississippian."
10. Shale and clay, "Hannibal Shale."
9. Limestone, not flinty, "Trenton and First Magnesian."
8. Sandstone, "Crystal City Sandstone."
7. Limestone, moderately flinty, "Jefferson City Limestone."
6. Sandstone, "Roubidoux Sandstone."
5. Limestone, very flinty, "Gasconade Limestone."
4. Sandstone, "Gunter Sandstone."
3. Limestone, not flinty, "Proctor Limestone."
2. Sandstone, "Lamotte Sandstone."
1. Granite, "St. Francis Granite."

The numbers express the order of occurrence of these beds as one passes outward from the central core of the region.

Stages in Ozark Erosion.

The geological formations in the Ozark region affect the kind of soil, as will be shown more fully below. The topography or shape,

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or character of surface, has likewise an important influence on the character of the soil. The shape that the region now has is the result of the forces of erosion or wearing down forces of rain, rivers, frost, etc., acting on the bowed up pile of rock layers described above. In general, that wearing off has had two main periods of work, the first one resulting in the wearing off of the original bowed up surface down to a lower and nearly level surface. The second period has eroded the existing valleys—the river valleys, the creek valleys and the innumerable hollows—in this nearly level surface.

When the first stage or period of erosion had been completed and before the second had begun the whole region was a gently undulating plain, a smooth country considerably lower than it is at present. The highest parts of it at that time were probably not more than 600 feet above the sea. In order to bring about the present conditions in such a region it had to be raised so that its rivers could dig deep valleys into this smooth country. This has been done and the old smooth surface has been cut by valleys so thoroughly that it is difficult to realize that it ever was smooth. The valley cutting has not been equal in the number or the depth of valleys, in all parts of the region, partly because the uplift that raised the region the last time was greatest along a line running from the vicinity of Iron Mountain to the southwestern corner of the state. The deepest valleys are therefore not on this line, for the streams here are small, nor do they occur on the outer border of the region because the uplift was not so great here. They are deepest about half way between the line of greatest uplift and the border.

Small parts of the old smooth surface exist at various places, such as the regions around Salem, Rolla, Lebanon, Springfield and on the high ridges between the main rivers.

The valleys, especially the larger valleys, are not simple. By that statement is meant that they do not slope uniformly down from the high plateau to their existing bottom lands. The descent is often made in two stages. The first descent is rather steep, less so than the second, but it is a well defined drop of 50 to 150 feet. From the foot of this slope a plain extends toward the river. It is smooth except where it is cut with valleys. It varies from a few hundred yards to several miles in width. It occurs usually on both sides of the stream. It is well developed along the Gasconade river from the southeast corner of Laclede county downward. It occurs also on Big river in St. Francois county and on most of the other large streams of the Ozark plateau belt.

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From the inner boundary of this bench there is another steep descent, steeper than the other, to the existing river or the bottom lands lying along it.

This descent is often precipitous. The other never is.

The inner border of the bench, next to the river, is thoroughly cut up into small valleys and hollows by streams, most of which head only a short distance back from the river, on the bench. The larger creeks, however, head back in the plateau beyond the outer boundary of the bench. These latter are the only streams that cut into the outer border of the bench since there are relatively few in number. The outer part of the bench is therefore much smoother than the inner.

The adjacent part of the high plateau, like the inner border belt of the bench, is usually quite thoroughly cut up with valleys and hollows.

There are therefore only about five essential features in the topography of the Ozark region. These are:

1. The remnants of the original plateau, produced by the leveling action of the wearing-off processes acting on the original upbowed series of rock layers.

2. The hill edges of this plateau where it borders on the belt of bench land.

3. The bench-land plains. They lie from 100 to 300 feet or even more above the existing valley floors of the streams.

4. The hilly belts along the edges of these bench-land plains and between them and the existing valleys.

5. The valley floors or flood-plains.

The old plateau may be entirely worn away from between two adjacent bench-land plains; it may exist as merely a string of knobs lying along the watershed between two adjacent bench-land plains; it may exist as a narrow but continuous ridge or it may still exist as a broad belt of high plateau.

The bench-land plains may coalesce across watersheds of adjacent river systems making a broad area, or they may be mere narrow ribbons lying along the streams but high up above the existing valleys. They may be nearly level, or may be cut up by ravines only along the edge next the present stream, or may be cut up across their entire width. In other words, they may be destroyed as plains and converted into hill belts by the ravines that have been cut down to or near the level of the existing valley floors.

The bordering hill belts may be broad or narrow, and the bottom lands along the streams may be wide or narrow. Notwithstanding these variations the topography of the Ozark region as a whole falls

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into the divisions given above and in its main features, therefore, is simple.

Of the smooth areas of the Ozark region the high plateau surface is the oldest, the bench land plains are next in age and the modern valley bottoms are the youngest. The hill surfaces are continually being slowly renewed. Their surfaces being sloping surfaces the soil is being continually renewed by having the old material washed off and newly formed material exposed at the surface.

The height of the Ozark region is nowhere sufficient to have any determining effect on the growth of crops. Any crop that will grow on the prairie lands surrounding the region will grow on the highest parts of the Ozark plateau where all other factors except elevation are made the same. The kind of soil is of more influence in this regard than is the small elevation. The gravelly and stony soils are well drained, porous, easily and rapidly warmed. They dry out and warm up in the spring earlier than the soils in corresponding latitudes either to the east or west of the Ozarks.

SOILS.

Their General Character.

Soils are made up of clay, silt, sand, gravel, stones, and vegetable matter. The clay, silt, sand, gravel and stones constitute what is known as the mineral or inorganic part of the soil. The vegetable matter constitutes the organic part. In agricultural soils the mineral or inorganic part is greatly predominant. The organic part makes up a very small proportion of the total weight of the soil. The inorganic part is the original part. The vegetable constituent has come from the partial decay of plants that have grown and died on and in the soil.

The inorganic constituents, and therefore the bulk of all soils, are derived from the breaking up or disintegration of rocks. The character of the soil depends greatly, therefore, on the kind of rock or rocks from which it has been derived. This is true not only from the point of view of physical character of the soil but also from the point of view of the plant food that it contains. The food used by plants in their growth is derived partly from the air and partly from the soil. Of the part taken from the soil the organic constituent supplies one part and the inorganic or mineral constituent supplies the rest.

The plant however cannot use the food until it becomes soluble in the water that is in the soil. Through the disintegration or decomposition of the rocks into soil the mineral elements of plant food are made soluble, not readily in pure water but in the soil water which carries in it certain acids derived from the air and from decaying vegetable matter.

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The minerals do not all dissolve at once, however. They dissolve only from the surface of the soil grains or particles, since the water can only get at their surfaces.

The greater the surface area exposed to the soil water, the more solution will be effected, providing other conditions are favorable. It is well known that there is a larger surface exposed in a given weight of very fine particles than in the same weight of coarse particles. The finer the soil particles, other things being equal, the more concentrated is the soil solution. There is a limit to the advantage gained by this however. That is reached when the particles become so small that the open spaces between the grains are not large enough to allow the water to circulate through the soil with sufficient freedom. The air also cannot get into such a soil to do its part in making the plant food soluble.

The rock material, after being changed from rock into soil, does not always retain the position it occupied as rock. It may have been carried away by various natural transporting agents, to a greater or less distance, and dropped in another place.

Soils that have been carried away from their place of origin are called *transported soils.* They may be of local derivation, like the alluvium of small creeks, or of far off derivation like the alluvium of large rivers or the soils of glacial or wind deposits. The alluvium of small streams is usually of simple composition, partaking only of the nature of the rocks of a small region which are apt to consist of a smaller number of distinct kinds of rocks than occur in a larger region. The alluvium of large rivers and the soils of glacial regions may be of very complex origin as well as composition.

Soils that retain the same location that they had as rock are called *Residuary soils* or *Sedentary soils.* They have a very close relation to the rock on which they lie though they may not be derived wholly from it or from a rock similar in character. In many cases the soil layer in any square foot of residuary soil may be what has been left from the wasting away of rock layers many times thicker than the soil layer. A few feet of soil may be what is left of many hundreds of feet of rock. In such cases several different kinds of rock may have been wasted away and the soil may have constituents derived from each. Nevertheless residuary soils are as a rule less complex in origin and composition, physically and chemically, than transported soils.

The soils of the Ozark region, with the exception of the alluvium of the creek and river valleys and a belt of varying width along its northern border, are residuary. The valley alluvial belts are usually narrow and from the point of view of area they are not of great importance. In point of production, however, not only per acre but

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total, they are very important. The rivers, with the exception of the Osage and White rivers, rise within the Ozark region so that their alluvium is of local derivation.

The belt of transported soil along the northern border of the region is probably the southward thinning edge of the glacial clay and silt layer which covers the uplands of northern Missouri like a blanket.

Soil Classification.

The terms in use by farmers such as clay, loam, sand, sandy loam, etc., are sufficient as far as they go but the soil to which these terms are applied vary widely in different localities. As used by farmers, these terms are not the result of accurate comparative study on a broad basis. They cannot be expected to be applied with any great degree of accuracy to soils in widely separated localities. Until wide areas of soil had been accurately studied by scientific methods no comparative and comprehensive knowledge of the soil existed, and under such conditions classification is impossible.

Various criteria have been used as bases for soil classification, such as the origin of the soil, the kind of rock from which it was derived, the crop to which it seemed best adapted and the natural growth found on the soil originally, such as the kind of trees or grass.

Each of these recognized one or in some cases more than one of the easily noticed differences in soils. The ideal classification would be one that would take into consideration as many as possible of the many different conditions affecting a soil, or characteristics possessed by it.

In order to describe soils intelligently and to deal with those from a large area of country where there are wide variations in soil character it is necessary to establish some facts or characteristics around which similar, though not identical, soils may be grouped. This grouping and such sub-grouping of the soils as may be effected on the basis of other characters of more restricted, though considerable, range constitutes soil classification. The characteristics around which they are grouped are recognized as the basis of classification. They (primary bases) must be common to a great number of soils.

Until within the last few years there has been in America no well defined and universally recognized basis of soil classification. Soils had not been studied in a systematic way. No great store of facts concerning them gathered from widely separated areas had been accumulated. The data were not at hand on which to build a soil classifica-

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tion. Before classification of any considerable number of things, facts or phenomena, is possible a rather intimate knowledge of such objects, facts or phenomena must have been accumulated.

The only system of classification used at the present time to any important extent in the United States is the one that has been developed by the Bureau of Soils of the United States Department of Agriculture. The primary basis of subdivision is Physiography—a combination, in a broad sense, of geology and topography. The bases for further sub-classification are in categoric order, Geological formation (kind of rock) from which the soil is derived, general size of the soil particles and finally the texture and color of the soil. Other factors enter as modifying factors and are recognized in the grouping, but the factors named are the principal ones.

CLASSIFICATION OF OZARK SOILS.

General Grouping.

The Ozark region in Missouri is a unit geologically differing in all respects from the country surrounding it except a portion of northern Arkansas. Its soils are all residuary except the narrow belts of alluvium along the creeks. It forms therefore a distinct group of soils, related, it is true, to soils in other parts of the country but not to those of the immediately surrounding country. The region differs also from the surrounding country in topography. It is therefore a physiographic unit. On the basis of its rocks and its topography its soils constitute a single soil group.

The rocks of the region as a whole are limestones.

Similar, or even identical soils, may be found in other states or in other areas in Missouri but these areas, except small portions of adjoining states, are cut off from the Ozark region by intervening territory with entirely different rocks, different character of surface and different soils. Although the Ozark rocks are chiefly limestones, the region, as stated above, is not entirely made up of such rocks. The limestones are not everywhere of the same character. The character of the country also is different in different parts of the region so that there are abundant reasons for dividing up the soils of the region into sub-groups.

On the basis of the character of the country, its rock beds and its soil the region is easily subdivided into three sub-regions:

(1) The Ozark Border.
(2) The Ozark Plateau.
(3) The Ozark Center.

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The Ozark Border.

The Ozark border is lower than the rest of the region as a whole. It is made up of a greater number of different kinds of rocks than the rest of the region and these rocks as a whole are less flinty than those of the rest of the Ozark region. As a whole the Ozark border is not so rough a region as the other areas. Every one of these characteristics is of profound influence in determining the character of the soil and especially its availability for agricultural purposes.

The Ozark Plateau.

The Ozark plateau differs from the Ozark border in having an entirely different group of rocks underlying it. Its rocks are more uniform in character, however, than are those found in the Ozark border belt. Were it not, therefore, for the different kinds of surface produced by the work of the rivers draining the region its soils would be rather uniform. The roughness or smoothness, and to a certain extent, the height of the different parts of this region determine the character of its various soils. This region comprises the area drained by the Gasconade river and its tributaries, the greater part of the Missouri area of the White river drainage basin, and a portion of the area drained by the Osage river.

The Ozark Center.

The Ozark center differs from either of the other two regions in the character of the rocks underlying its different parts and in its roughness. Its rocks differ widely from each other but the most of its area is underlain by very flinty rock. Its soil, therefore, is full of stones. It has two small areas, however, in which the amount of stone in the soil is very small. Taken as a whole, however, the Ozark center is a region of very stony soils, the Ozark plateau of moderately stony soils, and the Ozark border of slightly stony soils, though one of its areas has a considerable quantity of stone. The distribution of each of these areas is shown on the map accompanying this report.

Each of these areas is further subdivided into smaller groups of soils, according to characteristics having smaller areal distribution than the broad ones just described. The characteristic most abundantly used in the subdivision of the areas described above is that of character of individual rock series or layers. Some of the beds are sandstones, which decompose into a sandy soil. Some are limestone beds, free of flint, while others have a great deal. Some beds are nearly pure limestones, others have a great deal of clay. Another characteristic used is that of character of topographic detail. Very badly cut up regions are differentiated from smoother regions even though the soils

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of the two areas are derived from identically the same rock. They do not have the same productive capacity, agriculturally, and should not be grouped together.

The Ozark region as a whole, following the nomenclature suggested by the U. S. Bureau of Soils, is called a soil Province. This is subdivided into three sub-provinces: the Ozark Border, the Ozark Plateau and the Ozark Center.

Each of these is further subdivided into soil series or groups as shown on the soil map accompanying this report. Beyond this the differentiation is not carried on the map though the various kinds of soils in each series, or as many of them as are known, are described under the series in this report.

In the following pages the main soil groups are first described, followed by a description of the more important soils, so far as known at the present time, occurring in each group.

OZARK BORDER SOILS.

The Springfield Soils.

Distribution.

The main area of the Springfield soils lies in the southwestern part of the state including the greater part of the area covered by Greene, Lawrence, Jasper, Newton, McDonald, Barry, Stone, Taney, Christian, Webster, Dade, Polk and Cedar counties.

The belt of rock from which the soil was derived continues on around the Ozark region as may be seen by consulting any good geological map of the state. Between the northern part of Dade county and the northern part of Benton where the belt becomes buried by other formations, it is narrow. It appears again north of the Missouri river and extends eastward as a continuous belt to St. Charles county but it is not a factor of any importance in soil formation on account of the narrowness of the belt, and its covering of clay from another source. It forms a narrow belt from the Missouri river southward through St. Louis, Jefferson and Ste. Genevieve counties. This part of it, except a small part of the St. Louis county portion, lies close to either the Missouri, the Meramec or the Mississippi river. It is rather deeply dissected, therefore, and has very little tillable land in it. In St. Louis county it is extensively covered with glacial materials so that the soil covering is not typical residuary soil.

Along the southern and southeastern parts of the Ozark region the rocks that form the Springfield soils lie far south of the state line in Arkansas.

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Southwestern Missouri is typically therefore the region of Springfield soils.

Topography.

The topography of the main part of the area in southwestern Missouri is that of a plateau sloping gently to the northwest.

It is highest therefore along its eastern, southeastern and southern boundaries. It is considerably higher along the boundary than the adjacent country, to the east and southeast. Its slope to the northwest is gradual, however, while that to the southeast is steep. Small streams which, tributary to White river, drain its steep eastward slope have cut deep narrow valleys back into it for a few miles. The eastern boundary of the plateau has been made ragged by the ravines which have been cut back into it by the southeasternly flowing streams. For several miles back from its edge it is best described as a thoroughly cut up or hilly country. It consists of deep, narrow hollows and narrow, branching, stony, wooded ridges. Except where James river and its tributaries have cut a deep gash back into it, the width of this ragged border varies from five to ten miles.

Beyond the heads of these steep hollows the country becomes an even plain. This would be its character over the whole of the rest of its area were it not for the fact that this part also has creeks and rivers draining it and these have cut valleys into its surface. They are not so deep, however as those that drain its southeastern edge. Each of the rivers flowing across it, such as Spring and Pomme de-Terre rivers have made belts of hilly country along which they flow, but the hills are not so high, the valleys are not so deep and the slopes are not so steep as those along its southeastern border.

The eastern edge of the Springfield plateau, running northward from Marshfield stands slightly above the country east of it but not so high above it as the southeastern edge stands above its adjacent country. There is no river east of it as large as White river which lies southeast of it so that the ravines which drain it do not cut it up so completely as the southeastern border is cut up.

North of Cedar county the belt becomes narrow and its elevation less. It is not so high above the sea level and does not stand noticeably above the county lying east of it. Sac river has cut into it in Cedar, Dade and St. Clair counties and the Osage flows along it for some distance in St. Clair and Benton. They and their tributaries have cut the country up thoroughly but not deeply, so that a large part of the hilly land, along Sac river especially, is capable of cultivation.

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The Osage belt is a little rougher because the dissection is somewhat deeper. This hilly region, along both the Sac and the Osage is nearer like the hill land within the Springfield plateau than that along its southeastern border.

In Cooper, Pettis and Saline counties the belt widens again. Its topography in Saline and Cooper is hilly, in Pettis smooth. Cooper and Saline, however, are not even so hilly as Cedar and much less so than the eastern rim of the plateau in Webster, Christian and Douglas counties.

The smoothest land within this belt and the largest area of smooth land lies in the great southwestern expansion of the belt in southwestern Missouri. There is no other area of smooth land of equal size on this formation in the world.

The Rocks.

The Springfield soil area is underlaid by the Mississippian series of geological formations. These consist of the following rocks, beginning with the highest:

1. Chester Limestone.
2. St. Louis Limestone.
3. Keokuk Limestone.
4. Warsaw Limestone.
5. Burlington Limestone.
6. Chouteau Limestone.
7. Hannibal Shale.
8. Louisiana Limestone.

It will be noticed that all these rocks are limestone with one exception, the Hannibal shale. The area, however, is not evenly divided up among these several formations. The Chester formation barely occurs in the state so that for all practical purposes it may be left out of consideration. The same is true of the Warsaw limestone.

The St. Louis limestone has a very limited distribution in the state and still less in the Ozark region. The Keokuk limestone occurs only at two places in the Ozark region and in each of these its area is extremely small. The Hannibal shale barely occurs in the Ozark region and the Louisiana limestone does not have a typical occurrence south of the Missouri river. The Hannibal shale is the soil maker within the Ozark region only in narrow belts in Cedar, Dade, Webster and Wright counties. Practically all the residuary soils of the whole area, therefore, have been derived from the Burlington limestone, the others occurring only in small areas or in narrow belts. The Chouteau limestone has a wide occurrence in the Ozark border region but it is of practically no importance as a soil maker on account of its

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occurrence in narrow bands and its close association with the Burlington. The Springfield soils, therefore, are chiefly Burlington limestone soils. They are not all typical, however. It is only when they occur in broad areas that they occur unmodified. Where the area is narrow the soil is modified more or less by the next higher formation. This is the coal measure sandstone or sandstone and shale. Even in the large area of these soils occurring in the southwestern part of the state the true residuary Burlington limestone soils are covered on many of the high flat ridges and the prairies by soils derived from shaly and sandy rocks that originally overlay the whole region. These ridges are narrow, however, and the prairies small, so that the great body is typical.

Northward from this large area the character of the soil changes, but only in proportion to the amount of foreign material that is incorporated in it from other rocks. The Burlington limestone itself is very constant in character around the whole Ozark border. It is gray, rather coarsely crystalline, usually very highly fossiliferous and contains a large percentage of flint in nodules and bands. This latter varies somewhat in amount but it is so nearly universal in its occurrence in this limestone that it may be considered so. The soils are all more or less stony therefore, the flint of the rock remaining in the soil as stones instead of decomposing or disintegrating into soil. Unlike limestone soils in many regions, however, the soil layer, or rather the layer of decomposed material overlying the solid ledge rock, is thick. The ledge rock is rarely exposed except on steep slopes, the only exceptions being those localities where the soil is practically free from flint stones. Even in these places the limestone is rarely seen on smooth fields.

The Soils in General.

These soils are all silts of varying colors, running from black, through various shades of reds and yellow to white or gray. The proportions of clay and sand never or very rarely run very high in any of these soils. They are, therefore, usually mellow, or are easily made so with fair treatment. They are well drained, also, and are moderately early and warm. These characteristics are due in part to the greater or less abundance of stone or gravel in the soil. It is almost universal in its occurrence and in some of the types it is very abundant.

The subsoils are universally reddish in color. The intensity of the color varies somewhat but is everywhere greater than that of the soil. Like the soils they contain a considerable proportion of silt, very little sand, but also a considerably higher percentage of clay than the soils. The average percentage of clay in the soil runs from 10 to 20 and that of the subsoil is a little higher as a rule but rarely running above 20.

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Like the soil, the subsoil has a varying proportion of stones and gravel intimately mixed with the other constituents. Where the soil is extremely stony the subsoil is slightly less stony. Where the soil has very few stones the subsoil usually has a higher proportion. The stone content in the subsoil is more constant over the region as a whole than in the soil.

The natural growth on each kind of soil will be described under each separately.

The Soil Types.

In this general report which is chiefly a reconnoisance report it is thought unwise to undertake to give exact locality names to all the types of soil, and in the cases where names have been given to them such names must be regarded as merely tentative. If in the further prosecution of the work the results of the detailed study of the areas warrant the retention of the names used in this report, they will be retained, otherwise not. The subdivision into types in this report is made partly on the basis of the physical and chemical analysis, partly on the timber growth, partly on the presence or absence of considerable quantities of loose stones in the soil, on the geological formations, on the topography, the color and the performance of the soil under cultivation.

The Stony Black Oak Silt Loam.

This is the extreme variety of the stony soils of the series. The stones, always chert, range from 25 to 90 per cent of the total mass of soil. The finer part of the soil is a silt of essentially the same physical conformation as that of most of the other types of the series. There are two phases of this soil recognized by the farmers of the region. The "white flint" soil is made up of 40 to 90 per cent of small fragments of white apparently perfectly fresh chert or flint usually from 2 cu. in. in size downward. The fragments are always angular.

The "red flint" soil is made up of 20 to 80 per cent of soft, porous rough, reddish fragments of chert, of larger average size than those of the "white flint" phase but usually not more than 6 to 8 inches in diameter with an average size somewhat less than that. The fragments are roundish due to weathering. They are never rounded as a result of having been rolled. The proportion of stones to fine part in the soil in this phase is smaller than in the other phase. The less stony parts of each phase are sometimes cultivated. The "red flint" land is considered the best. It is more fertile, lasts longer, and stands drought better.

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The origin of the two phases is partly topographic and partly due to the original character of the rock from which the soils were derived. The "white flint" soil occurs on most of the southwardly facing slopes, especially if they be steep, while the "red flint" occurs on the north slopes. On these the evaporation is less and the rocks are more moist, oxidation and decomposition are more active. The cherts or flints as well as the limestone are more thoroughly decomposed. On the southwardly facing slopes moisture is soon evaporated and decomposition is retarded. On the other hand changes of temperature are more rapid, so the flint, being compact, is broken up into small pieces by the rapid warming and cooling. In such cases the differences are undoubtedly due to topographic position. Often the same narrow ridge will have one phase of soil on the south slope and another on the north.

In other cases these different phases occur in positions where the differences cannot be ascribed to the slope or exposure to the sun, but are probably due to the nature of the flint that was in the limestone before it was converted into soil.

The subsoil is a reddish, stony, silty clay essentially alike in both phases. The timber growth on this type of soil is chiefly black oak. On the northwardly facing slopes and in deep narrow hollows where evaporation is slight and the soil is usually more moist than elsewhere, red oak and white oak are most common. On the very stony ridges black oak is the most common tree. Scattered post oak and black jack trees occur also. On the southward slopes the trees are all scrubby as a rule with post oak and black jack rather common. There is no undergrowth and at the present time not much grass. Until the timber grew up dense enough to shade the ground there was a thick dense growth of the native blue stem grass. It has been smothered out by the shade and at the present time its place is taken by a thick growth of annuals and perennials, many of them being native legumes.

In portions of southern Stone and Taney counties the short leaf yellow pine has occupied much of the southward slopes and many of the dry stony ridge tops.

This soil is found in the hilly belts of the areas of the Springfield soils. These belts, as stated above, are the southern, southeastern and eastern border belt and a belt along each side of all the larger creeks and rivers within the area. Not all of the areas of these hill belts, however, are included in this type. Most of the other types of the series occur in small areas here also, but this is the dominant type. It is impossible to outline these areas on a small map. A map on a scale of at least one inch to the mile will be required and their location can only be determined by detailed work.

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The width of the border belt will average fifteen miles along the southeastern part of the area and about five miles along the rest of the boundary. Along the streams the width will vary from one to five miles.

A small proportion of this soil is in cultivation. No exact statistics are at hand but it is estimated at not more than ten per cent. In the rougher parts of the border belt there are areas of several hundred square miles where not more than two or three per cent is in cultivation.

The physical analysis of the fine part of this soil is about as follows:

SOIL.

Clay	12 to 14%
Silt	60 to 65%
Sand	15 to 20%

SUBSOIL.

Clay	13 to 15%
Silt	60 to 68%
Sand	12 to 18%

No chemical analysis of a sample of the extreme phase of this type has been made because of the difficulty of collecting the samples. The sampling augur will not penetrate it on account of the great abundance of stones. Some analyses of stony phases of other types of the same series, derived from the same rocks and occurring in much the same condition as these show a low percentage of humus, nitrogen and phosphoric acid a moderately good percentage of lime and a high percentage of potash.

The proportion of humus is low both because of the open porous and dry nature of the soil which favors the rapid burning out of the humus by weathering and because of the autumn fires which have spread over these hills from time immemorial. Before the timber grew dense enough to smother out the grass with shade the autumn fires burned the dead grass. Since that time they have burned the leaves. No vegetable matter has been allowed to accumulate on the land. There is no layer of mold that is gradually changing to humus, nor has there ever been.

It is not easy to supply rapidly the humus under cultivation on account of the difficulty of plowing effectively among the stones. It is rather difficult, on that account, to get vegetable matter thoroughly mixed with the soil.

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This soil is not adapted to the growth of the general farm crops on account of the difficulty of cultivating it and its low fertility. Its less stony phases, however, are well adapted to the growth of clover and other legumes. These plants "catch" readily and do not die from drought badly nor are they subject to freezing out. The land is so rough however, that a hay crop cannot be harvested easily. Clover fields can be utilized for pasture, however, with excellent results. White clover grows well and spreads rapidly especially during wet years. It is rather easily affected by drought.

Bluegrass and the other sod grasses do not grow well partly on account of the lack of humus and nitrogen in the soil. There are indications, derived from observation on this soil that the growing and pasturing of clover for a few years will enable blue grass to take hold. The great abundance of clay and silt in the fine part of the soil ought to make it an ideal soil for grasses of all kinds. The poor growth of grass cannot be due to the coarseness of the soil but is more probably due to the low percentage of humus. This can be supplied by proper handling.

The bunch grasses, especially orchard grass, grow quite well on this soil. They grow much better, however, after some humus has been supplied.

A great deal has been said about the fruit industry on this soil. There is no doubt that fruit trees will grow on it and will produce good fruit but it will never be possible to devote all or even a large proportion of these lands to the growth of fruit. The fruit crop is uncertain in that region on account of winter freezing and late spring frosts. It is extremely unsafe to depend on fruit growing alone as an industry. As a branch of general farming it can be made to pay.

The existing area of fruit culture can and will be extended in this soil but a point will soon be reached where the utilization of the best of it for pasture and the poorest for the growth of timber will be found more profitable than fruit growing.

The salvation of the area lies in a combination of fruit culture and grazing with the growth of legumes as important pasture plants. A farm on land of this soil alone, could not be a very profitable one. When it is owned in considerable bodies, however, and once set in clover and other grasses it can be used to great advantage as pasture land on farms which include some areas of the other soil types of the region, especially the bottom land soils on which hay and grain can be grown for feeding. Such pasture lands are cheap, fencing materials are cheap and when once set in grass and clover they will support almost as much stock per acre as much higher priced land in other parts of the state.

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Handled in this way, the less stony phases of this soil can be made profitable. A rotation in which a large reliance is placed upon legume crops and in which grain crops are not grown too frequently is to be strongly recommended for this class of soils. For the more stony portions the most profitable crop that can be grown on them is timber. It is not a soil for the small forty-acre farmer except in the immediate neighborhood of the towns or a railway station where the growing of small fruits can be engaged in with a moderate degree of profit.

Whether this soil will grow alfalfa or not is a question that has never been settled. It is quite probable that alfalfa could not be started on it without heavy fertilization but where properly prepared there is no sufficient reason yet shown why it should not grow, at least on the less stony places. The roots could penetrate to great depth and the clayey nature of the subsoil would hold water through prolonged drought. There is no real hardpan under this soil.

The Black Oak Gravelly Loam.

The finer parts of this soil do not differ essentially from that of the preceding type. They are both derived from exactly the same kind of rock and through the action of the same processes. They are both residuary upland soils. They differ in their topographic location, their proportion of stone and the ease of cultivation. The Black Oak Gravelly Loam varies in color from place to place from gray to yellow, brown and red. A greater proportion of the area of this soil has a reddish color than that of the preceding.

The proportion of stone, above a cubic inch in size, is considerably less than in the preceding type, rarely running higher than 15 to 20%. It is not so high as to make it unprofitable to haul the stones off the land. This is often done and the land made much more easily tilled. The amount of stony material less than one cubic inch in size varies from five to 40 per cent, running higher and lower only in rare cases. The proportion of stony material is about the same in the soil and subsoil.

The fine material of the soil consists of about 60% of silt, 10 to 15% of clay, and about 10% of sand, though in places the proximity to localities where the overlying sandstones of the coal measures occur modifies the amount of sand in the soil layer considerably. It does not affect the subsoil, however, to a great extent. The percentages of clay, silt, and sand in the latter are about 25 to 30, 45 to 60 and 5 to 15 respectively.

The timber growth on this type of soil is Black Oak and Red Oak to the practical exclusion of every other variety of tree growth

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except occasional White Oaks which occur on the northwardly facing slopes. Like the timber everywhere on all the soil types of the Ozark region, that on this type has grown up since the occupation of the region by white man and the stopping of the prairie fires. Before that time there was a growth, often very dense, of hazel and sumac brush. Just as such land is now described as "Black Oak land," a large part of it was formerly known as "hazel and sumac" land. At the present time occasional sugar maple trees and a few shrubs or small trees are usually found on the northwardly facing slopes.

There is no large area made up wholly of soils of this type. In general, the areas are small and more or less isolated. They occur chiefly in the hill belt of the Springfield series area, that is, in the southern and southeastern border belts and in the river and creek hill belts of the interior. They are closely associated therefore with the stony Black Oak soils. They occur, however, in the smoother parts of these hilly areas higher up the streams than the latter soils and on the gentler slopes. They do not occur, as a rule, on the flat topped ridges however narrow they may be. They are hilly or rolling land soils. They are derived from massive nearly pure limestone beds which carry a small amount of flint. In decomposing or disintegrating into soil, therefore, there is not a large amount of flint to be left in the soil. The same beds of limestone underlie much of the area of the more flinty type but only on steep slopes where the flint from higher beds which carry a much greater proportion of flint, has drifted down over the whole slope and made it all stony. The gravelly soils occur on the more gentle slopes on which the creep or drift from above is not sufficient to affect the amount of flint in the soil.

It is impossible at present to give the details of distribution of the soils of this type. To do that will require accurate detailed mapping.

A much larger area of this soil is in cultivation than that of the preceding type. It occurs more often on northwardly facing slopes and is better adapted to cultivation for that reason alone than the southwardly facing slopes. It is regarded as a strong soil in comparison with the other upland soils of the region though the physical and chemical composition of its fine material is practically identical with that of the preceding type. This latter, however, although a strong soil, is not cultivated to any extent on account of its content of stone. What was said concerning the crops that may be grown on the stony black oak soil applies here also. For the first few years after clearing it will produce fairly good crops of corn and wheat but on account of the small amount of humus in the soil to begin

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with this kind of cropping soon reaches the lower limit of profitable production. The small amount of humus is soon burned out and the nitrogen used up. Its store of mineral fertility, however, has not been seriously reduced by these few years of cropping and the soil being naturally a legume bearing soil, the growth of a few crops of clover or cowpeas, especially if one or two crops are turned under, brings it back to better condition than it had to begin with. This kind of farming, however, cannot be kept up indefinitely, for by it no mineral matter is given to the soil and considerable is being taken away in the crops of grain and clover. The soil as stated above is low in phosphoric acid and the time will come relatively soon when it will be necessary to add phosphoric acid to the soil.

Like the stony type of soil it is better adapted to grazing than to grain or hay raising on account of the unevenness of the land and on account of the stone in the soil. The physical condition of this soil, where it is treated with any sort of intelligent regard for the maintenance of its fertility is almost ideal. The fine material is almost wholly silt and clay but it contains so considerable an amount of gravel of all sizes that it is kept in a mellow condition. In cases where the land has been cultivated in corn for several years in succession and the humus all burned out it is inclined to bake when dry. When it reaches this condition it will, if abandoned or allowed to lie fallow for a few years, become covered with a dense growth of young sassafras brush.

Although both these soils occur in the hilly area of the Springfield soils yet they do not wash badly as a rule. The only reason for this is the presence of the stone and gravel in the soil. These enable the soil to absorb the larger part of the rainfall rather than to cause it to flow off on the surface. The stones act as obstructions to the flowing water also and hinder washing in that way. It is only where the amount of stone and gravel is very small that there is much loss of soil from washing. The streams of the region are clear excepting for a very short time during freshets. When ditches are started in the fields they are easily stopped by being filled with stones.

The Black-Jack Gravelly Loam.

This is a gray, brownish yellow to yellowish silt with a varying proportion of stone and gravel. The soil at the surface is usually gray but changes to a yellow or yellowish red color in a few inches, becoming continually a more decided red downward. The gray part of the soil rarely extends to greater depth than ten inches, and is usually less. The subsoil is some shade of red or yellowish red, being more often reddish than yellowish though not usually so deep

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a shade of red as the subsoil of the two Black Oak types already described.

The proportion of stone varies greatly, more than in either of the Black Oak types. In places the stone constitutes from 50 to 60 per cent of the total soil material and as a rule occurs in large angular masses as well as small fragments or gravel. In fact some of the most hopelessly stony areas of the whole Springfield series region belong to this type. The flint masses are large, sometimes more than a cubic foot in content, white, angular and undecomposed. The other extreme is that in which the soil is practically free from stone of any kind larger than a cubic inch or two in size. Rarely is this soil free from the latter and it usually occurs in quantities amounting to more than 10% of the bulk of the soil. Where the large stone is absent the small stones or gravel do not make cultivation especially difficult.

A physical analysis of a sample of the fine material of this soil from McDonald county showed 23% sand, 64% of silt and 11%clay, while the subsoil from the same locality showed 21% sand, 49% silt and 24% clay. This is probably not far from an average composition of this soil. The percentage of sand in this sample is probably a little higher than the average in this soil.

This soil type includes the area in which the principal tree growth is the Black-Jack Oak or Jack Oak or Black-Jack and also the gravelly parts of the small gray soil prairies of the southwestern part of the state within the Springfield soil area. The Black Jack brush has encroached rapidly on these prairies since prairie fires were stopped by man except where the land has been placed under cultivation. Black Jack is almost the only tree that grows on this soil. An occasional Post Oak and an occasional hickory may be met with.

This is one of the two main types of soil in the Springfield area that occur in the smoother parts of the region. It occurs on the broad gravelly prairie areas, on the somewhat rougher land on the boundaries between the prairies and the hill belts and on narrow, gravelly ridges.

This soil occupies, therefore, with the one next to be described, the central part of the Springfield area. It is confined almost wholly to the western side of the Ozark region and is found chiefly in the southwestern counties. It does not occur in its typical form on the eastern or northern side of the Ozark area.

A large part of the area of this soil is now in cultivation. When the uplands began to attract the attention of farmers this soil was the first to be put under the plow. This is due not to its greater fertility than that of the types described above but because of the smoothness

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of the country and the lack of a heavy growth of timber to be cleared off. It is in fact universally regarded as poorer land than that of the preceding types but is much easier put into cultivation and easier to cultivate when once broken out. Its growth of timber was never dense. It is much more so now, where not cleared and in cultivation, than ever before. Probably about 70% of this area is now under fence and most of what is cleared and broken out is under the plow.

The physical analysis shows that this soil is adapted to general farming. Its chemical analysis shows, like all the other soils of the Ozark region, a low percentage of nitrogen (.118%), phosphoric acid (.055%), and of lime (.137%), and a high percentage of potash (—), and of magnesia (.171%).

It is devoted to the growth of wheat and corn chiefly—in fact almost exclusively—with an occasional crop of oats or clover.

On account of the smoothness of the land farm machinery can be run over it and its yield of wheat is fair so that it is devoted to the growth of that cereal more than to any other. The next most important crop is corn. It yields under favorable conditions of season and tillage and while the humus content is still fairly good, about 35 bushels of corn and 10 to 15 bushels of wheat. Of late years farmers have been using considerable bone meal with wheat, increasing the yield up to 20 bushels in good years.

It is not regarded as a good clover soil. It does not catch so well as on the Black Oak types and does not grow so rank. Not nearly so much attention is paid to growing it as on the latter types. The soil is, however, fully as much in need of it as any of the other types and sooner or later it will have to be done or else the growth of other legumes will have to be taken up. Phosphoric acid will also have to be added in time unless more attention is paid to live stock husbandry and all manure carefully saved and put on the land.

Grass is not grown to any extent on this soil. It is rather too dry, loose and open to be especially adapted to such a crop. The main thing, however, that makes the growth of grass unprofitable seems to be the lack of humus in the soil. In a few localities where this has been supplied, blue grass gradually takes possession and forms a good sod but does not grow so rank as it grows on the clay lands of the northern part of the state, except where an abundant humus supply has been derived from clover.

Orchard grass and the other bunch grasses do quite well. The southern grasses such as Bermuda have not been tried on this soil. There is very little doubt that the growth of a few crops of clover or cow peas, turned under, will supply the necessary constituents for the successful growth of Blue grass. When this is done the continuous

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cropping of grain crops can be stopped and the land still handled profitably.

A larger proportion of this soil is devoted to the growth of fruit than that of either of the other types described. This not due to its greater adaptability to the growth of fruit trees or plants but rather to the fact that it is an upland soil and a larger proportion of it lies adjacent to the railways. The fruit growing localities are determined largely by the location of transportation lines. It is generally agreed that fruit trees do not grow so well on the upland prairie and gravelly Black-Jack soils as on the Black Oak gravelly soil.

The danger from late spring frosts is not so great on the upland as in the valleys. The fruits grown on this type of soil are apples, peaches and strawberries. A crop of the latter is a little more certain than of the former and they usually find a good market. The industry is large enough to enable the product to be shipped in carload lots. The chief losses have resulted from heavy rains at the time of harvesting. Other small fruits do well but no effort has been made to grow them for the market except locally.

Of the two tree fruits mainly grown in the region, apples are more extensively grown and are more profitable. The peach crop is made very insecure on account of spring frosts. Apples are often injured also though a total failure is rare, so also is a full crop. The latter probably once in six to eight years, the former once in ten.

The area devoted to fruit or that is likely to be devoted to fruit in the future is small proportionally. The bulk of the area, therefore, will be devoted to general farm crops and to live stock husbandry. To make this permanently successful it is absolutely necessary to get the soil in the condition to grow grass. This can be done by putting humus and nitrogen into the soil and keeping it there. The easiest way and the cheapest way to do this is by the growing and plowing under of clover or cowpeas—not by the harvesting of a crop of hay and turning under the stubble but by turning under the whole crop. For land that is to be put to grass one or two crops turned under will be sufficient if the grass be pastured. When the land is to be devoted to grain or hay growing this will have to be done, in the case of grain growing, every three or four years, or with hay every six or eight years. Where grain crops are grown continuously even with clover in the rotation, it will be necessary before long to apply phosphorus in the form of some mineral fertilizer as well as return all manure to the land. Lime is another thing that will benefit this soil in places but it must not be thought of as a fertilizer nor as a material that can take the place of the clover and phosphorus fertilizers. All three of these things are or will soon be a necessary con-

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dition for the growth of profitable grain crops on this soil. No one of them can supply the requirements. The lime can be obtained easily and cheaply in the vicinity, the clover can be grown. The only thing that will cost more than labor and seed will be the phosphorus.

The Post Oak Silt Loam.

This is a gray bluish or yellowish stony to stone free soil with a bluish to yellowish or grayish stony to stone free subsoil. It is largely a silt, the fine material in the soil giving a percentage of 13 for clay, 69 for silt and 15 for sand while the subsoil gives the percentages of 31, 53 and 14 for clay silt and sand respectively.

The stone constituent of this soil is chiefly "chunk" rock, consisting of stones varying in size from 5 to 10 inches in diameter with a very small percentage of gravel. In many cases the gravel constituent is absent leaving a stony to stone free silt.

The subsoil is, as stated above, bluish to yellowish or reddish yellow or mottled though rarely as red as the subsoils of the Black Jack type. Its stone constituent is somewhat greater than that of the soil though not so great as that of the other types. The proportion of gravel is about the same as that of the soil down to a depth of from 24 to 30 inches, below which it usually increases. Where the soil is stone free the subsoil, at least down to about 24 inches is apt to be free also and of a rather tough blue clay.

The timber growth on this soil is almost exclusively post oak. It graduates into the gravelly Black Jack land on the edges, the latter tree appearing in small proportion at first and gradually increasing.

Before the occupation of the country by white man the area of this soil type consisted of oak openings, or open grass covered areas with few trees of large size, exclusively of post oak. The ground was full of roots, however, which sprouted up every spring and were regularly killed down every fall by fires. When white man stopped the fires these sprouts grew rapidly, except where the ground was cultivated, and have produced a very dense growth of tall rather smooth saplings almost exclusively of post oak. Many of the old trees still stand and rise above the tops of these young oaks. The annuals growing on this land originally consisted chiefly of grasses. Legumes were not entirely absent but they were much less abundant than on the gravelly types.

Since the growth of young trees became sufficient to shade the ground the grasses have been killed out so that now the ground is bare or supports only a few weeds. The leaves, however, are burned every autumn so that the store of humus is not being increased.

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This is an upland soil. It occurs on the flatter areas of the country, on the flat topped ridges, the flat, poorly drained areas or "flatwoods" of the native nomenclature, and in the flat sag-like valleys on the uplands—usually around the heads of prairie sloughs. The total area of this type of soil is smaller than that of any of the types already described. There are very few large bodies made up exclusively or chiefly of it. Its occurrence is usually in small patches in sags and on flat ridges where the drainage is poor. Like the Black Jack soil area it occurs almost exclusively in southwestern Missouri, and is more abundant in Webster, Greene, Lawrence and Barry counties than elsewhere. North of Dade county it is hardly known and along the northern and eastern sides of the Ozark region the Springfield belt is narrow, near the Mississippi river and too well dissected for the occurrence of any significant areas of this soil.

A rather smaller proportion of the area of this soil is under cultivation than of the preceding type. It has the advantage of being free of gravel and occasionally free of stone but the greater disadvantage of being cold and poorly drained. It is considered the poorest of the tillable upland soils of the region. It is rather hard to work and requires a favorable adjustment of many factors to produce good crops. At the present time it is farmed just like the other upland soils of the region. It is devoted to the general farm crops, which in this region consist almost exclusively of corn and wheat. In some cases these post oak areas are converted into permanent timothy meadows, but little attempt has been made to improve the land by manure drainage or other means except an occasional application of commercial fertilizer. It produces fairly good crops of timothy and red top hay but poor crops of corn. The dryer portions, the stony portions with yellowish or mottled subsoil will produce fair crops of wheat but the stone free phase with the blue clay subsoil produces very poor crops of wheat except in rare cases when the seasons are dry.

Clover does not grow well on a large part of this soil. It will produce fair crops in favorable seasons on the better drained stony areas but on the blue clay subsoil areas it will not grow profitably under existing conditions.

The dryer areas will produce good crops of cowpeas and they will grow in the other parts so that there is no reason even with these soils for buying commercial nitrogen nor for leaving the soil forever barren of humus.

This type of soil has not been studied very much but the indications are that it is badly in need of nitrogen, humus and lime. These can all be supplied cheaply by the farmer himself. One sample from this type has been analyzed chemically and the result showed a low

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percentage (.179%) of nitrogen, a low percentage of phosphoric acid (.04%), a low percentage of lime (.149%) and a high percentage of potash (.27%).

These results do not differ greatly from those of the preceding type though the percentage of phosphoric acid is somewhat lower. The defect seems to lie in the physical character, in the poor drainage, the dense nature of the subsoil, and the low percentage of sesquioxides of iron present. It must be borne in mind, however, that these figures are for a single sample only and no broad generalizations can be based on them.

The farmer of this soil as well as some of those on the Black Jack soil report the occurrence of a hardpan from one to three feet below the surface. No systematic study of these occurrences has been made but in the few cases studied nothing but a stiff or tough clay layer was found except that in some of the Black Jack areas the stone, a few inches below the surface, became for a foot or so more abundant and more closely compacted but no place has yet been found where such stony layer or clay layer was cemented into a definite impervious hardpan layer.

The flat areas of the Post Oak belts with the blue clay subsoil will be improved with drainage. Whether this will have to be done by open ditches or by under drainage cannot be stated here. An application of lime (600 to 1000 pounds per acre) and the growth of a few crops of cowpeas would bring this soil into much better condition. Fruit trees seem to do about as well on this soil as on the preceding type. Not a great deal of it however, is devoted to growing fruit. Strawberries are not grown on it to any extent, the dryer gravelly Black Jack soils being much better adapted to them.

The Bottom Land Loam.

This is a gray to black loam or silt loam, rarely passing into a sandy loam, with or without gravel. It is everywhere of an open, friable texture having sufficient clay and silt to enable it to hold moisture but not enough to make it difficult to cultivate. It is free of stone of large size, larger than an inch or two in diameter except where a ravine or hollow from the hills opens out into an area of level bottom. In such cases, the wet weather streams have washed stones from the hills and spread them out in cone fashion around the mouth of the hollow. The gravel in the loam is always more or less rounded and oxidized to a reddish color.

The subsoil is either a reddish clay or gravelly clay of that color. The gravel is usually in layers and lenses alternating with clay, and rarely or never cemented into an impervious layer. There are on

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most of the larger streams two or more benches or terraces, usually two well defined ones. In such cases the upper bench has a clay subsoil with a small amount of gravel down to three or four feet while the lower bench, the modern alluvium, usually has a gravel or gravelly clay subsoil.

The timber growth on this soil consists of white, red, burr and pin oak, sycamore, walnut, elm (two species), hackberry, wild cherry, butternut, white and black hickory, mulberry, sassafras, persimmon, pawpaw, and a rather long list of shrubs and vines, all of which grow luxuriantly. The more gravelly parts of the bottom lands were devoid of large trees and grown to sumac and hazel brush until the occupation of the region by white man.

These soils are confined to the creek and river bottoms. They lie, therefore, in long narrow strips. They are rarely more than half a mile wide and from that down. The average width of the alluvial strips is about three-eighths of a mile for the region as a whole. The narrow bottoms of the small hollows in the hills where there is no stream, except during heavy or protracted rains, are not included here, since the bottoms of such valleys are mere long narrow strips of soil that is but a little more than a bed of loose stones.

Practically the whole of the area of these soils is in cultivation and has been so for many years. The first farms of the region were made on them. The lands where they occur have always been the highest priced and most profitable lands in the Ozark region, and are especially well adapted to general farming. They produce good crops of corn, wheat and clover. Oats is grown to some extent, but the yield is not enough to make it a paying crop as a rule, the reason probably being climatic. The yield of corn, when a corn-wheat-clover rotation is run, and the soil handled carefully, will average about forty bushels per acre, running in favorable years to sixty or more, while it rarely drops below thirty. The yield of wheat under the same conditions will average twenty bushels except where it is cut down by rust or climatic influences.

Clover yields one and a half tons of hay and from one to four bushels of seed per acre. Blue grass does not grow luxuriantly, partly because no attempt has been made to set it and none is likely to be made. The land is too valuable for growing grain. The gravelly silt soil is rather loose, also, for the sod making grasses. Orchard grass where it has been tried has done well. These bottom lands will probably never be converted into permanent pasture. This will be located on the adjacent hill land. The profitable farm in this region, therefore, will include at least a small area of bottom land for grain growing and a larger area of upland for pasture and clover hay.

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The water supply in these valleys is abundant and of good quality.

With a rotation of corn, wheat, clover, the turning under, rather than the burning, of the waste and the return of the manure made from the feeding of the corn and clover on the place, back to the land these soils will produce grain crops indefinitely with the addition of mineral fertilizers occasionally.

The Bolivar Soils.

The Rocks.

The soils included under this name are derived from rocks of the lower carboniferous age lying just beneath the limestones which form the soils of the Springfield series. They are the Hannibal shale and sandstone. They occur as clays, clay shale and fine-grained sandstone of a prevailing yellow to greenish yellow color. Since the shale is much more abundant than the sandstone, the soils are more often clays than sands. The sandstones are very fine-grained and earthy.

The average thickness of these rocks is only 30 to 50 feet. The width of the belt of country in which they constitute the surface rock is narrow, often not more than a mile, though occasionally amounting to twice or three times that width. From the areal point of view, therefore, they are not very important rocks. They usually outcrop on slopes which are capped by the limestones which form the Springfield soils. The soils overlying them, therefore, are rarely derived wholly from them. Soil from the limestones has usually drifted down over them so that the surface soil is often considerably modified and only the subsoil is typical Bolivar soil material. On the other hand, the clays from these rocks sometimes extend out over flat areas as a thin layer of soil with a subsoil made from underlying rocks of a totally different character. These two main modifications of these soils occur on the outer and inner borders, respectively, of the belt.

The Soils in General.

These soils are prevailingly of a somewhat cold clayey nature, and where they occur over an area of considerable width the country is usually flat. This emphasizes their tendency to wetness. Except where stones have drifted down over them from near-by areas of Springfield soils they are wholly free from loose flint stones. Where the sandstone is well developed, small boulders of soft earthy, yellowish, perforated sandstone may occur. Where the subsoil is derived from the next underlying formation on the inner border of the belt it is stony. The timber growth on this soil is, on the more clayey

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portions, chiefly laurel oak and post oak. The post oak grows on the flat areas and the laurel oak on the gentler slopes and in the small valleys. On the sandier parts black oak and red oak are the important trees, though black jack is not uncommon. On the bottom lands laurel oak with the common bottom land trees such as elm, walnut, cherry, etc., are found.

These soils do not form a continuous ring around the Ozark region. Like the Springfield soils they are southwestern Missouri soils and occur chiefly in Webster and Polk counties. A narrow belt extends into Hickory county as well as into Cedar, but they disappear before reaching the Osage river. They do not reappear again in the whole circumference of the Ozark region.

They are practically unknown south of the Springfield-Memphis line of the Frisco railway. The rocks producing them gradually thin out southward and, although they extend south of the railway, they are so thin that they do not influence the soil. They begin as a narrow belt near Cedar Gap and extend northward in a rather narrow belt immediately east of the Springfield soils through the country in which Marshfield, Fairgrove, Pleasant Hope, Tremont, Bolivar, Polk and Elkton are situated. Beyond this they rarely occur, but they are found in narrow belts along Sac river and its larger tributaries in Cedar and Dade counties. The belt is not continuous, unbroken and of even width along the course described. It is cut by streams as well as by eastward projections of the Springfield series and is broken by outlying islands of the latter. It is broader, but more broken up, in Polk county than anywhere else.

Timber Growth.

The timber growth consists of mixed oaks, post oak on the flat areas, red and black oak on the better drained areas and laurel or shingle oak in the wettish upland sags.

The Soil Types.

Only two general types of soil are known to belong to this group. *The Polk silt loam* is the upland soil of the group. It is gray to yellowish in color, free from loose stones and on account of the general smoothness of the topography in which it occurs is nearly all in cultivation. It is rather heavy and intractable, but under favorable conditions will produce fair to good crops of wheat and corn to which it is largely devoted. The wheat stubble fields in the autumn are usually covered with a growth of *Spanish Needle*. It is in need of drainage on the flatter areas and in the upland laurel oak sags which are rather abundant.

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The Polk Loam.

This is the bottom land soil of the area, but very little of it occurs. The streams practically all cut through the bed of shale and into the underlying cherty limestone. The alluvial soils partake therefore of this latter rock to a considerable extent. The Polk loam occurs unmodified only in very narrow belts along the small streams. It is dark to black in color, rather sticky, with a silty to fine sandy subsoil.

There are doubtless small areas of fine sandy soil derived from the fine sand beds of the shale, but such areas were not seen.

The Perryville Soils.

Distribution.

The Perryville soil belt or ring is, like the two just described, only a partial one, not extending entirely around the whole Ozark region. It lies, however, on the eastern side of the Ozark region rather than on the western.

The Rocks.

It is underlaid by limestones belonging to a wide range of geological ages, from the lower part of the Ordovician to the Devonian, all alike, however, in being rather pure, chert-free limestones. The soils are therefore chert-free silts and clays, either red, gray or some shade of yellow or brown depending upon the formation from which they have been derived and the amount of leaching to which they have been subjected. The formations, beginning with the lowest, is a gray subcrystalline limestone of Lower Silurian age which weathers to a rather yellowish soil. It is about 100 feet thick and of essentially uniform character from top to bottom.

The next formation is the Trenton limestone, which has two divisions, a bluish fine grained crystalline lower half and a coarser crystalline gray upper half. Both weather to reddish soils. The next, which is a group of several beds belonging to the Upper Silurian and Devonian ages, consists of beds of limestones of varying character, all of them chert free, however. There are also a thin bed or two of shale.

These formations all lie along the eastern part of the Ozark region extending from the Missouri river in the western part of St. Louis county southward to the lowlands of Cape Girardeau county. They dip eastward beneath the rocks making up the Springfield soils, such of them as occur along the eastern side of the Ozark region. This belt of rocks leaves the Missouri river in the western part of St. Louis county. Through St. Louis and Jefferson counties the belt of their outcrop is very narrow and the soil overlying it has been modified to

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such an extent by the adjacent Springfield soils that it does not differ essentially from them and has been included in that group. The typical Perryville soils do not appear north of Riverside, where the Mississippi river turns to a southeastward course. From this point southward the belt widens very slowly continuing as a narrow band entirely across Ste. Genevieve county. At the south line of the county it widens rapidly and occupies a broad belt across the eastern part of Perry and the central part of Cape Girardeau counties. In Ste. Genevieve and Jefferson counties, the rocks forming the Springfield soils lie between the Perryville belt and the Mississippi river. In the southern part of Perry and all of Cape Girardeau county the belt is cut off from the Mississippi by the intervening belt of Wittenberg soils.

Topography.

The topography of that part of the belt of country underlaid by this soil which lies north of the latitude of Ste. Genevieve is rough. The belt is narrow and lies close to the Mississippi river. It is rather high also. This has resulted in its complete dissection. The soil is everywhere badly eroded and in most places the top soil is gone leaving the subsoil only. In fact, in places the rock is entirely bare.

South of the latitude of Ste. Genevieve, as the belt widens it becomes smoother. Over its whole area in Perry county, except a belt along the river a few miles in width, it is a rolling plain, becoming somewhat hilly along the creeks. The creek hills are rounded, rock beds being rarely exposed and the uplands have many gentle undulations due to sinks in the underlying limestone. The chief factor controlling the value of this soil for agricultural production is that of topography. Where the land is relatively smooth the soil is well adapted to general agriculture. It differs in this respect from the Springfield soils where a factor of considerable importance is the quantity of loose stones in it which determines its agricultural value more often than any other factor.

This whole region, uplands and valleys, was covered with forest when white man first saw it. The luxuriance of the forest growth was one factor in attracting settlement. It was one of the first upland areas of the Ozark region that attracted settlement and the upland farms on it are among the oldest upland farms of the state.

In geological formation, topography, physical constitution, color, origin and fertility, the soils of the greater part of this area are practically identical with the soils of the limestone belt of the great Appalachian valley in Pennsylvania, Maryland, Virginia, Tennessee and Alabama. The Bureau of Soils of the U. S. Department of Agriculture has named these soils the Hagerstown Soils.

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The Soil Types.

There are, so far as our studies up to the present time have enabled us to classify the soils of this belt, two types of soil in it. These are the Perryville silt loam and the Perryville clay loam. As the region is studied further other types will probably be found. Our classification into two types only, is an extremely broad, simple one that is evident to every casual observer and is based on a very hurried study. This belt has received less study than any other belt of its size in the Ozark region.

The Perryville Silt Loam.

This is a reddish brownish or gray silt loam, having about 8 to 15 per cent of clay, 65 per cent of silt and 10 to 20 per cent of sand. It is practically free from loose stone, such as do occur being fragments of limestone as a rule. Fragments of chert or flint are rare. The surface drainage seems to be sufficient except on small areas where former sink holes have been filled up. It is mellow, easily cultivated and under proper treatment, produces good crops.

The subsoil is a reddish silt differing from the soil chiefly in its deeper red color and a somewhat higher percentage of clay. There is no sharp boundary between soil and subsoil. The soil section gradually changes from the paler color of the soil to the deeper color of the subsoil accompanied by a gradual increase in the percentage of clay. Analyses of the subsoil show a percentage of clay varying from 10 to 20, of silt varying from 55 to 70 and of sand varying from 18 to 22, depending upon the depth. The clay increases and the other constituents decrease in depth.

The subsoil rests directly on the bed rock and usually at a depth less than 30 feet. The limestone, being nearly pure carbonate, is decomposed only by solution, so that its surface always presents the appearance of fresh rock. Except where the occasional shaly beds underlie the soil there is no gradation from one to the other, though the lower layers of the subsoil contain many detached masses of limestone.

The Perryville silt loam, being free from gravel, stones, and coarse sand is a cooler soil than those of the Springfield series. Its humus burns out more slowly under the same treatment, yet on account of the cropping system of practically every farmer on it, its supply of humus is low at present. This type of soil is farmed almost exclusively by Germans and has been farmed by them for a great many years. The few exceptions to this rule do not affect the cropping system because the system of the majority determines it. The Germans in Missouri are grain farmers. The live-stock farmer among them is extremely rare. They have practically taken possession of the lands on the eastern and northern borders of the Ozark region. This is a region

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of stone-free silt and clay soils, and a rolling hilly topography. Their system of exclusive grain farming has reduced the humus content of these Ozark border soils to a very low per cent and has also caused the soils to wash badly. The two redeeming features of their methods of farming are their careful use of the little barn yard manure that they get and their rotation of clover with the grain. This, however, is not sufficient to supply the necessary humus, nor is it conserving the supply of mineral plant food, especially phosphorus.

The forest trees growing on this soil are laurel, red, pin and white oaks, hickory, walnut, cherry, post oaks, hackberry, elm, maple.

There is relatively little timber still standing that is of much value. In the more rolling parts there is still some good white oak timber and everywhere there is enough red, pin and post oak to supply local demand for fuel, posts and the rougher building material.

The exact distribution cannot be given. It can only be stated that this soil occupies the smoother parts of the belt as described above. In Ste. Genevieve county it begins two or three miles north of Zell church, is only about a mile in width and runs thence south to the Saline creek breaks. After an interruption of a few miles along the hill belt of the creek, it appears again south of the creek and soon spreads out so as to include the whole east central belt of Perry county and the central belt of Cape Girardeau county. In these latter counties it is interrupted only along the creeks. It is the soil of the rolling uplands. Probably ninety to ninety-five per cent of this soil is in cultivation and has been in cultivation for nearly a quarter of a century—some of it much longer than that. An uncleared area on the rolling upland is a rare occurrence.

Along the slopes, in the ravines and hollows, there are still plenty of trees, but on the smoother lands they have long since been cut off.

The fact that this soil has been selected to so great an extent by a race of grain farmers is of itself an indication of its adaptability to these crops. This is not the only factor or possibly not the chief one that caused the settlement of the Germans on this land, yet it is certainly one of considerable importance.

The main crop is wheat. The exact proportion of the area in wheat to that of the total area of plowed land cannot be stated but it is approximately 40 per cent. Corn comes next with probably 25 per cent, and the other crops follow, clover being the most important.

Fertilizers are not used to any significant extent. The yield of wheat will average 12 bushels and of corn about 30, though in favorable years it will run five or ten bushels higher.

This is one of the best soils of the Ozark region. With proper handling its productive capacity can be easily maintained for an indefinite period. It is probably low in its phosphorous content, but with

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a proper system of farming that supply will suffice for almost an indefinite period. It may be increased, however, by the addition of commercial phosphates which on this soil, under intelligent direction may be done profitably.

Its physical character is excellent and its location good. The system of farming now adopted is not calculated to maintain its fertility. It is absolutely essential that more humus be added, that the nitrogen supply be maintained or increased and that the exhaustion of the mineral fertilizers by continuous wheat crops which are sold off the land be stopped. In other words, a system of live-stock farming must be adopted, the grain grown on the land must be fed back on it and a rotation with more grass in it must be established.

During the last few years some of the farmers have begun the growing of cowpeas because of the increasing difficulty of growing clover. They are cut off for hay, however, rather than turned under and do not supply enough organic matter to the soil. The growth of clover is becoming more and more difficult throughout the whole Ozark Border German belt, due to the exhaustion of the humus and possibly the phosphorus and lime of the soil by continuous grain cropping.

Whether this soil needs lime or not is not known. That is a subject, however, worth investigation.

The Perryville Clay Loam.

This is the surface soil made from the subsoil of the silt loam where the latter has been eroded away. It is the hill land soil of the region, therefore. It is of a reddish color except where its exposure has been long enough to weather it to a gray by leaching out the iron oxide. It is sticky, tough, rather difficult to work, gray yellowish or red in color and usually shallow. There is very little change in character, down to a depth of three feet. The subsoil is a little deeper red in color and has a slightly higher per cent of clay.

Ledge rock is often exposed, sometimes in the fields, while in many cases the fields occupy the narrow ridge tops, extending down the slopes on each side to where they become so steep that both soil and subsoil have disappeared and the bare rock is exposed. Fragments of limestone are not uncommon, though they are too scattering to be of any value in preventing the washing of the soil. They are usually larger than the average size of flint fragments found in Ozark soil.

The natural growth on this soil is red, black, post and white oak, with a varied assortment of shrubs and brush. Post oak is the most abundant tree with red and black following and white oak last. The flatter and more eroded places as well as the more thoroughly leached places support post oak. It never grows on the red colored soil, if the

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color is a decided one. Chinquapin oak is often found where the soil is shallow and the bed rock frequently exposed.

This is the hill land soil. It occupies the tops of narrow ridges and the slopes of the badly dissected part of the belt. It occurs in irregular belts along the creeks and a rather large area lies along the heads and near the heads of the short streams flowing into the river. It does not extend entirely up to the river, being separated from it by a belt of brown loam soil whose origin is not yet certainly determined, though it is probably a river or glacial deposit. It lies, however, between this and the valley lands of the silt loam area.

A much smaller proportion of this soil is under cultivation than of the silt loam. The percentage will not run higher than 40.

It does not produce heavy crops of anything. The same methods of farming are carried on as on the silt loam. It is devoted almost exclusively to grain growing with a little higher percentage given over to the tame grasses, usually timothy. Wheat is the chief crop and corn the next. The yield of wheat per acre is not more than 10 bushels, while that of corn, except where the land is manured heavily, is not more than 25. It is better adapted to wheat than corn. Cowpeas grow moderately well but are not grown to any important extent.

The two things that this soil needs above all others is vegetable matter and nitrogen. Its supply of mineral fertilizers is not less than that of the silt loam. The vegetable matter will lighten the soil, and make its water holding capacity greater as well as remedy its extremes of wet and dry conditions which are now a serious drawback. The nitrogen will supply the plant food that is now deficient.

Both these can be supplied cheaply and effectively by the plowing under of cowpeas or clover. The growth of these crops and their harvesting as hay will not affect the result because no more humus nor nitrogen is added than is taken away in the crop.

The next most effective way is the addition of barnyard manure in large quantities, but this is impossible for the whole of these soils simply because it cannot be obtained.

The next best method is the plowing under of all sorts of, and every crop of, weeds and the addition of commercial nitrates, which will probably be unprofitable to the man who undertakes it.

A slower way is to convert all this land into pasture.

The Hillsboro Soils.

Distribution.

The next ring of rock and soil coming inward toward the center of the Ozark region is also an open ring. It also lies along the eastern and northern part of the Ozark region and does not

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appear on its western or southern side. Along the northern part of the region, also, it lies north of the Missouri river, so that for our present purposes that part of it need not be considered. It runs from the Missouri river in the extreme western part of St. Louis county southward through St. Louis, Franklin, Jefferson, Ste. Genevieve, Perry and Cape Girardeau counties. It barely touches the northeastern corner of Bollinger county. It is bounded on the east by the Perryville group belt and on the west by the belt next to be described.

The Rocks.

It is underlain by a sandstone which retains its characteristics rather well along its whole course. The bed is rather thin, never exceeding 150 feet, dips eastward and outcrops usually in a rather steep slope that faces westward. The actual width of outcrops of the rock is often less than a quarter mile and rarely more that half a mile. The sand, however, which determines the character of the soil occurs in a belt much wider than the width of the rock belt. The sandy soil belt has its eastern boundary on the eastern boundary of the rock belt, but its western boundary is further west. The rock originally extended further west and in being worn back to its present position it left considerable sand in the soil. Sand also has been washed from the stone and spread out over the smoother land immediately west of it. The amount of sand in this belt varies considerably becoming less and less westward. The subsoil in going westward across the belt departs more and more from the subsoil of the typical sandy soil and partakes more and more of the nature of the next belt of soil to the west.

The width of the belt of sandy soil, where it leaves the Missouri river is about five miles. In the vicinity of Hillsboro it is about ten miles. Through the northern part of Ste. Genevieve county it is rarely more than two or three miles and in Perry it is not prominent on account of the thorough dissection of the belt. In the northern and central parts of Cape Girardeau county it is about five miles in width.

It continues southward and ends against the northern side of the Southeast Missouri Lowlands.

Topography.

Only the northern and southern ends of this belt are utilized to any extent for agricultural purposes. From the Missouri river southward to the northern part of Ste. Genevieve county a considerable portion, probably about 40 per cent, is in cultivation. It is a rather hilly region but the valleys are not so deep and so abundant as to leave no land smooth enough for cultivation. Through the greater part of Ste.

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Genevieve and Perry counties, on the other hand, the country is so badly cut up with narrow valleys and ravines that it is practically useless for anything but timber growing and for pasture. The bed of sandstone also seems to be rather thin along this part of its belt so that it is not a prominent factor in soil formation. The ravines are cut deep into the rock beneath the sandstone leaving nothing but sharp-topped ridges to be occupied by the sandy soil. This washes easily so that in most places it soon disappears. South of Apple Creek, however, the country is considerably smoother, and the belt of Hillsboro soil, although quite narrow, is occupied to a considerable extent with farms. The largest area of this soil that is cultivated lies in Jefferson county. It extends northward into the eastern part of Franklin, also. In Ste. Genevieve and Perry counties it is mostly hilly and uncultivated while in Cape Girardeau it is again cultivated.

Timber Growth.

On account of the fact that the sandstone bed whose distintegration has produced this soil outcrops in a narrow belt barely more than a mile in width with a steep westward slope, the greater part of this soil belt lies west of the outcrop of its parent rock. The soil lies therefore on a rock of a different kind. This affects, more or less, the nature of the subsoil. This is true particularly of the western and northern parts of the belt. It is less true for the eastern and southern parts. In the western part of the belt, therefore, the surface soil is sandy, while the subsoil has a higher percentage of clay and is often stony. The roots of trees reach downward through this thin sandy soil and into the stony clay subsoil. On account of this the timber growth is essentially the same as that on the soil next to be described. It consists of black, red, white, and post oaks, with scattering hickory on the upland.

On the eastern side of the belt where both the soil and subsoil are derived from the parent sandstone the soil is dryer and the timber growth consists chiefly of scrub oak.

The Soil Types.

There are two main types of soil in this area, an upland type and an alluvial type. The latter is of rare occurrence. It never occurs unmixed with material from other soil and rock belts because of the elevation at which the country lies with respect to the level of the valley bottoms and the narrowness of the belt.

The Hillsboro Loam.

The soil varies from a loam through sandly loam to an almost pure sand. The distribution of these phases of the soil depends on the dis-

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tance from the outcrop of the sandstone and the local topography. The loam lies along the western side of the belt and on slopes where the sand has become mixed with some of the clay from the underlying rocks. The sandy loam occupies the middle part of the belt and the sand occurs in patches chiefly in the eastern part of the belt. The loam usually has a rather clayey, sometimes stony subsoil, the other two phases have more sandy subsoils.

A sample of this soil collected in the northern part of Ste. Genevieve county showed 26, 60 and 11 per cent of sand silt and clay, respectively, in the soil and 28, 45 and 26 per cent in the subsoil. A chemical analysis of a sample from Jefferson county contained a low percentage (0.073 per cent) of nitrogen, a low percentage of phosphoric acid (0.952 per cent), a good percentage (276 per cent) of potash and a low percentage (0.312 per cent) of lime.

One of the most noticeable features of the country in the Hillsboro belt of soils is the somewhat ragged appearance of the fields due to gullying. The topography is rolling to hilly. The soil is very friable. It washes into gullies easily. Where not carefully handled the fields are pretty badly washed by the time the stumps are out. It is especially liable to receive poor treatment. It is not naturally a very fertile soil. It does not occur in large bodies of smooth country where it can be cultivated easily. It does not respond with a bountiful yield and great profit to thoughtless, careless treatment. Furthermore the larger area of it lies in Jefferson county, where it is too far from St. Louis to be of great value as a truck soil and not far enough to overcome, with its rather moderate promises of reward, the stronger attraction of the city. The young men leave the farms to the old people, or to renters. It is not a soil that can stand the average renter very long.

It is not a hopeless soil, however, by any means. It is warm, porous, easily, though not cheaply cultivated, has no hard pan, has sufficient clay in its subsoil to hold a good store of moisture, does not leach excessively, is no poorer in the elements of fertility than many other more highly cultivated soils and grows clover and other legumes luxuriantly. A soil with these characteristics is not hopeless. It can easily, with intelligent cultivation, be made the home of a great many prosperous farmers.

The crops now grown on it are corn, wheat and clover. These are the crops to which it is best adapted and it will grow them with profit providing it is not allowed to wash badly and its supply of humus is kept up by plowing under a crop of clover occasionally. Not more than one crop of corn should be grown in a rotation period of about four or five years.

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The Union Soils.

Distribution.

The Union belt of soils lies next to the Hillsboro belt on the west and runs parallel with it. Like the latter, the Union soil belt extends around only the eastern and northeastern part of the Ozark region. It begins in Bollinger county on the northern boundary of the southeast Missouri lowlands. Its boundaries are not sharp lines, there being a gradual change eastward into the Hillsboro soil and westward into the Reynolds belt. The latter, however, is sharper than the former. The line is very crooked, however, being driven eastward by east-west hollows and westward by similarly trending ridges. In general its western boundary runs northward a few miles west of Zalma and continues thence about due north to the southwestern corner of Ste. Genevieve county. It runs thence northwestward by Ulam, Weingarten and the northwestern corner of the county. Thence it passes by Vineland, Frumet, Lone Dell to Moselle. It then turns southwestward and follows approximately the line of the St. Louis and San Francisco railway to the vicinity of Rolla, where it turns north, then northeastward, and finally, after much turning, reaches the Missouri river a few miles west of Washington in Franklin county. Throughout Bollinger and Perry counties it is about ten miles wide. In Ste. Genevieve it is narrower, becoming very narrow in the southwestern part of Jefferson county. After turning westward its width is considerably greater though very irregular.

The Rocks.

The rocks from which this soil is derived, consist of magnesian limestones with a rather small amount of flint. The layers are usually rather thin, and there are occasional dark to greenish shale layers. As a rule the rocks are fine grained and are rather impure. The impurity is clay. This gives the rocks, with their fine-grained character, a rather dull earthy appearance and a rather smoothish fracture. Granular crystalline beds occur interbedded with the others, but they constitute a considerably smaller proportion of the rocks than the other. When these rocks disintegrate into soil, the lime is dissolved out and carried off in solution in the streams, and the clay is left behind. There being a rather small proportion of flint, there is not much of that material left as stone in the soil.

The Soil.

The soil and subsoil consist, therefore, of nearly stone-free to moderately stony limestone silt and clay with a gray to yellowish color, passing downward into a yellowish to pale reddish clay subsoil. The

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subsoil is rarely deep red. Where the land is level the soil may have a very dark or nearly black color.

Topography.

The whole of this belt of country is a rolling to hilly region. The greater part of it lies along watersheds, or is crossed by small streams only. Castor river runs within it for a few miles only; Whitewater and Crooked Creek dissect it to some extent in Bollinger and Madison counties, but neither cut deep enough to render any considerable part of the county unfit for agricultural purposes as a whole. A considerable portion, about 30 per cent, is made too rough to cultivate but these areas grow timber or grass. In Ste. Genevieve county it is badly cut up and a considerable portion rendered unfit for agriculture. A belt across it on either side of the Meramec also is rather rough, but only moderately so, so that a considerable portion of it is in cultivation. Elsewhere it is gently to strongly rolling or moderately hilly.

Timber.

The whole of this area is timber land except where cleared and in cultivation. The principal trees are white, post, red, laurel and black oaks on the uplands and the usual Ozarkian bottom land timber in the valleys. White oak and post oak, the latter predominating, make up the larger part of the timber. White oak is abundant on the northward facing slopes and on the yellowish clay hills. Post oak is the dominant tree on the more level-topped ridges and is mixed with the white oak on the narrower ones. Accompanying the white oak on the northwardly facing slopes are red oaks while black oaks usually accompany the post oaks on the flatter ridges, especially where rather well drained. They occur with the white oaks also, especially on the southerly slopes. Laurel oak occurs only in the northern part of the region and in moist, gently sloping valleys and sags.

The Soils in General.

The soils of this region are stone-free to moderately stony silt to clay soils, either gray or yellowish in color. The proportion of stone in them is least in the southern part of the belt and greatest in the northwestern part. The small amount of stones, the fine grain of the soil and the rolling character of the ground are all favorable to soil washing. With the exception of a narrow belt lying along the St. Louis and San Francisco railway it is practically all subject to erosion. This keeps the top soil pretty well washed off, and does not permit the accumulation of a layer of humus-rich soil on the surface, when treated

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in the ordinary way. The rather fine grained soil does not permit as rapid burning out of the humus as is the case with the more stony Ozark soils but it is rather easily washed off. Under careless management it does not accumulate. In this respect this belt does not differ much from the other Ozark soils. It is not a region, however, of old, worn out, gullied and abandoned fields. The land that has been cleared is all cultivated with more or less care. One reason for this lies in the fact that it has been almost entirely occupied by German farmers. While they are not very successful in increasing or even maintaining the humus supply of the soil they do not allow it to wash into gullies. There is a great deal of loss of soil due to washing but gullies are not allowed to deepen indefinitely.

Probably not more than 40 per cent of the total area of this belt is in cultivation. A considerable part of it can never be cultivated on account of its steepness and the shallowness of the soil. Probably about 60 to 70 per cent of the area can be made to produce either grain or grass. The rest will grow timber more profitably than anything else.

The upland soils of the whole area do not differ widely from each other. The most striking difference is in the proportion of stone. There seems to be no persistent regularity in its distribution so that no more can be said about it than has been said above. The soil of the western part of the southern area and of the southern part of the western area of the belt are apparently poorer than the eastern and northern parts respectively. They are the parts also most nearly free from loose stones. There are two possible reasons for this. The former soil lies only on the tops of high ridges while the lower slopes belong to the next belt of soil to the west. It has occupied this position a long time and has been badly leached. A large part of its mineral fertility, and especially its lime, has been leached out of it. On the other hand the soil along the eastern and northern parts of the respective areas of the belt are continually being freshened by the erosion away of the leached soil and the exposure of a more recently formed lower layer. The second possible reason for this difference of soil lies in the possibility that the better soils lie adjacent to the Hillsboro belt and may be lightened up somewhat by a small amount of sand. This, however, is a rather doubtful possibility.

The Crops.

The crops that are grown on this soil are the usual grain and hay crops. The acreage of wheat and corn on the upland is apparently about equal. The yield of wheat is 8 to 20 bushels, the latter only after clover or with bone meal, and of corn on the uplands about 30 bushels. Clover can be grown with reasonable success on all this

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belt except the most thoroughly leached lands. On these cowpeas grow well.

This soil is not naturally so fertile as the Perryville soil and occupies a more broken country, has a larger percentage of stones and is paler in color except locally where crystalline limestone layers occur. It has an even greater deficiency of humus, phosphorus and lime and is more subject to erosion. It is naturally stronger however than the Hillsboro soil and has been more considerately treated. Its greatest need is humus. The next is nitrogen and the next phosphorus. As stated many times already it is necessary to purchase only one of these, the phosphorus. The rest can be grown.

The Owensville Soils.

Distribution.

There are two general areas in which this soil occurs in bodies of considerable size. The easterly one occupies the highest part of the Ozark border plateau lying east of the Gasconade-Osage hill belt. It lies in Gasconade, Franklin, Maries and Phelps counties, chiefly in the former. The westerly area lies in a smilar topographic position on the west side of the Gasconade-Osage hill belt. It lies in Morgan, Northern Miller, Moniteau, Cooper and Benton counties.

The Soils in General.

This is a series of loam soils white, gray or black in color with a clay subsoil. The subsoil is reddish to brownish at top changing downward to bluish and at 24 to 30 inches to a yellowish mottled color. The thickness of the soil layer down to the reddish subsoil runs from 6 to 16 inches where best developed. It is underlaid by a sandstone in many places yet it is rather low in sand and what sand it has is fine sand. The sandstone bed underlying it is not continuous, but its lack of continuity is due to erosion so that if the soil were wholly residuary it would be sandy. Along the breaks where the typical area of this soil grades off into the wholly residuary soil there is a considerable percentage of sand and in some places a very sandy soil. This fact as well as its occurrence indicates that it is an outlying area at the southern edge of the Kansas glacial soil much like that of northeastern Missouri though no granite or crystalline material has been found in it. In many places it is underlaid by a bed of well-worn chert pebbles though this layer seems to be very patchy. In other places it is underlaid with a layer of very fine clay, some sand and a great many small polished though not well rounded chert fragments. The same material is found in small patches in several other places on the northern bor-

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der of the Ozark region and is known to extend southward as far as Cuba and possibly to St. James. The Owensville region, however, is the only one where it occurs over a large unbroken area of any considerable size.

Another universal characteristic of this soil is the occurrence in it of a great many small round black iron concretions about one-tenth of an inch in diameter. The grayer the soil the more abundant are these "buckshot."

Topography.

The topography is everywhere smooth, varying from a very gently undulating to a somewhat strongly rolling or faintly dissected plain. The western area has a larger proportion of smooth land than the eastern one.

Timber.

The greater part of this area was, until the beginning of the 19th century, either prairie or very open woods. At present the central flat ridge is still prairie though brush is rapidly encroaching on all of it that is not cultivated. The fringing woods are largely post oak. In those places where the land is flat it is almost exclusively post oak. Where the drainage is better it has a sprinkling of red oak, black oak and hickory. There are three general types of this soil sufficiently distinct to be easily made out by a hurried examination.

The Owensville Silt Loam.

This is the original uneroded surface soil or at least the least eroded surface soil in the region. It occurs on the prairie at Owensville extending westward nearly to Canaan and eastward and northeastward for five miles. There are also small patches of it in the vicinity of Drake. It is a gray to black loam with about 15% of clay, 50 to 60% of silt and about 20% of sand. The clay percentage in the subsoil as a whole from the top of the reddish layer downward is 20 to 30. A chemical analysis of a sample taken a mile west of Owensville gave a fair percentage (.176%) of nitrogen, a low percentage (.057%) of phosphoric acid, a very high percentage (.449%) of potash, a low percentage (.215%) of lime and a high percentage (.688%) of magnesia. It is a good wheat and timothy soil and raises fair crops of corn. The flat areas need drainage and the heavy cropping with wheat is exhausting its supply of phosphorus. Its supply of organic matter is fair but the soil would be better if it were increased. With good treatment, a legume in the rotation and phosphorus added in bone meal or phosphate rock the soil will last indefinitely.

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The Lane Silty Clay Loam.

This is apparently a phase of the Owensville soil that is a poorer soil, probably due in part to erosion and in part to original character. It is less loamy in character, the whitish layer with small polished gravel is nearer the surface and the soil is grayer in color. Its fertility is much lower, judging from the farm houses and crops as seen on a hasty trip. It is a poor wheat as well as corn soil, except where by erosion it has become mixed with the residuary soil from the underlying limestone.

It occurs in a considerable body lying north and south along the Bourbeuse-Gasconade watershed, chiefly west of the Owensville area which lies east and west along the Bourbeuse-Missouri watershed. Its extreme northward point lies only a few miles south of Hermann while a small isolated area lies at St. James on the Gasoncade-Bourbeuse watershed and apparently another at Cuba. It is probable that detailed work will disclose many isolated areas along the Frisco railway east of St. James as well as elsewhere. The typical area is on Lane's prairie in the eastern part of Maries county. In most places except on Lane's prairie it is covered with a dense growth of small post oak saplings with scattering black oak and hickory. Until the country was occupied by white man the whole area was either prairie or else was occupied only by scattering post oaks. These still exist in many places making a marked contrast with the younger generation which covers the land with a dense thicket.

The soil contains about 12% of clay, 60% of silt and 25% of sand, based, however, on an analysis of a single sample. The subsoil has a higher percentage of clay, is rather tough and close. It does not differ essentially, so far as physical analysis indicates, from the Owensville silt loam but its growth of post oaks and its lower crop producing capacity differentiate it from the latter with sufficient clearness. It is the more leached fringe of the Owensville soil and, if it be of glacial age, most of it was deposited at a greater distance from the ice than was the Owensville silt. A chemical analysis of a sample from Belle showed a low percentage (.029%) of phosphoric acid, a medium percentage (.201%) of nitrogen, a medium to rather low percentage of potash and a fairly good percentage of lime.

The clay or silt layer carrying the smooth chert fragments is exposed in many of the roadside ditches in Lane's prairie but it is rarely seen on the Owensville (Douglass') prairie. Both this and the Owensville silt loam are entirely free from stone. Wheat is the principal crop on the Owensville soil while hay is grown on the Lane soil. A casual examination fails to show a great amount of difference between these two soils except that the latter soils do not appear loamy.

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The farmers in the country say that there is considerable difference in their producing capacity.

The Owensville Stony Silt Loam.

This is merely a connecting link between the Owensville and Union soils. Running out on the long watershed ridges that radiate from these high plateau-like prairies there are long strips of a gray buckshot silty soil with more or less flint in it, underlaid by a yellowish more or less stony subsoil. The latter is undoubtedly residuary while the former seems to be a remnant of Owensville silt that has not yet been eroded off of these ridges. It extends southward as far as the St. Louis and San Francisco railway.

The Osage Soils.

Distribution.

This soil occupies a hilly region lying between the western edge of the Owensville plateau and the eastern edge of a similar plateau lying in Morgan and Miller counties. The northern side of the Osage soil belt extends to the Missouri river and the southern side lies a few miles north of the Rock Island railway.

The Rocks.

The rocks underlying it are magnesium limestones, much like those underlying the Union soil belt though they are somewhat finer grained and have a smaller proportion of flint. The soil, therefore, is almost entirely free from flint fragments. Along with the clay that has resulted from the decomposition of these limestones there is a greater or less amount of the Owensville silt and clay that originally extended over this region before it had been cut to pieces by the valleys and ravines that now traverse it. The proportion of this latter material is probably small except on the few areas of rather high flat country.

Timber.

The timber growth consists of a much larger proportion of softwood trees than any other soil area yet described except the Perryville. The most abundant trees are red oak, white oak and hickory with walnut, hackberry, elm, cherry, coffeebean, chinquapin oak, and a great variety of dogwood viburnum, thorn and other shrubs. On the dry ridges and southerly slopes the timber is sometimes scrubby.

Topography.

This is a very hilly region. It is the gateway through the Ozark border for the exit of the Osage and Gasconade rivers from the in-

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terior of the Ozark region. Both these rivers have cut deep valleys and their innumerable small tributaries have thoroughly dissected the country for some distance on either side of the main stream. The area extends from the central part of Gasconade county on the east to the central part of Miller county on the west and makes a rather long northwestern projection through Cole and Moniteau counties into the extreme northeastern corner of Cooper county. It is the roughest part of the Ozark border with the exception of small areas in Jefferson and Ste. Genevieve counties and the southeastern border of the Springfield plateau.

The Soils in General.

The upland soil consists of a stone-free, grayish yellowish or brownish silt loam. A strip along the northern boundary of the area has soil that approaches the Union soils in character while the soils in a similar strip on the south grade into the Vienna and Lebanon soils. They differ from the Union soils in their paler color, more clayey and less silty-texture and in the rougher topography in which they occur. They differ from the Vienna and Lebanon soils in being much less flinty and having a more yellow color. They cover the country rather uniformly even on rather steep slopes. The rock beds are frequently exposed but not over wide areas. In addition to the silty soil underlying much of the larger areas of the country there are occasional small areas of stony clays. These are small, are not cultivated and do not need description.

The Gasconade and Osage rivers have strips of bottom land of the finest quality but they are rarely more than a quarter of a mile in width. These bottom lands are all in cultivation.

This area of country is occupied almost exclusively by German farmers. The small inland towns have a decidedly German appearance with substantial and well-preserved but plain dwellings, gravelled streets, heavy stone walls and excellent brick or stone churches.

The same crops are grown here as on the Union and Perryville soils. Clover until recently was one of the main crops but during the last five years it has become increasingly difficult to grow it. It doesn't catch well and dies out rather easily. This is probably due to the exhaustion of the organic matter and lime. Probably clover would grow if these deficiencies were supplied.

The Howell Soils.

Distribution.

The only member of the Ozark border soil belts that extends across the southern boundary of the state from Arkansas into Missouri consists of a stony red limestone silt or silty clay stretching across Ripley,

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Oregon, Howell and parts of Ozark counties. The eastern end of the belt lies on the edge of the southeast Missouri lowlands. The western end lies against the rough stony gray lands of the central part of Ozark county and its northern boundary is defined by the southern edge of the rough stony land along Eleven Points and Current rivers.

Topography.

The country is rolling to hilly. In Howell and the southwestern part of Oregon counties the country is rolling. The valleys are shallow and the slopes are gentle. In the Eastern part of Oregon the belt is crossed by the Eleven Points river where it is very rough. Between the Eleven Points and Current rivers it is moderately hilly and another hilly belt lies along the latter stream. The northeastern part of Ripley and the adjacent parts of Butler and Carter counties are rather smooth.

Timber.

The whole region is covered with timber except where cleared. The timber growth is chiefly black and red oak with small amounts of white and post oak.

The Soils in General.

The upland soil has a reddish to gray color and the subsoil is deep red. The upland soil is covered with a great abundance of loose flint stones. Only very rarely is it stone free. Unlike most of the other stony soils of the Ozark region however the stone in the Howell soil lies largely on the surface. Beneath the surface there is not nearly so much of it. This is due partly to the fact that the stone constituent of all soils gradually works to the surface and also, and to a greater degree, to the fact that this stone did not come from the rock that made the greater part of the soil but from an originally higher flint layer. The flint was not so abundantly distributed through the rock but there was once a thick layer of solid flint whose position was above the existing surface, that has been broken up and the fragments left on the surface. There are still left a few high hills in the region, rising considerably above the surrounding country, that are capped by the original flint layer. The top of the hill at Sterling is one; King's Mt., east of Willow Springs, is another and the high hills in western Howell and around Spring Creek in Ozark are capped by it. Elsewhere it has been broken up by the wearing down of the country and the fragments only left on the surface.

There are four principal types of soil in this group. They are the (1) Gray Stony Loam, (2) Brown Silt Loam, (3) Gray Silt Loam, (4)Alluvium.

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The Gray Stony Loam.

The stony upland soil is a grayish silt at top changing to brownish and then reddish within a few inches from the top. Like all of the other residuary soils of the Ozark region there is no definite line between the soil and subsoil. The former becomes more red in color gradually and more clayey in character downward. The soil cannot be defined as the grayish top of the whole soil layer since this is only a very few inches thick in some places and several feet thick in others depending upon the amount of leaching that has gone on.

The soil is very low in organic matter and full of stones.

The timber growth consists of red and black oak, black jack, post oak and scattering hickory and white oak. On the southward slopes it is scrubby. It is nowhere very large.

On account of the stony nature of this soil only a small part of it has been put into cultivation. Along the railway considerable areas of it have been planted to fruit trees but elsewhere a large part of it is still in timber.

The Brown Silt Loam.

Scattered over the surface in many places are small sags or basins lying from 5 to 20 feet below the surrounding rim with a stone free or nearly stone free soil. They vary in size from a few hundred square feet up to several acres. Most of them are small, too small to become important factors in the soils of the county. They are usually surrounded by very stony land lying somewhat hummocky, the stone being more abundant on the hummocks. These small details of the surface occur chiefly in the more level parts of the region. They are the results of the unequal solution of the underlying limestone. The small basins are places where the limestone has dissolved more rapidly and allowed the surface to sink below the general level. The fine parts of the soil from the surrounding higher ground have then been washed into the basins. The stones in the basin are covered by it. On the rim of the basin and the hummocks of higher ground the finer parts of the soil have been washed out and the stones are left more bare than usual. There are areas of considerable size where the surface is chiefly a succession of these features. Other areas occur, especially near the streams, where their occurrence is rare. The same soil occurs in the rather broad round bottomed shallow valleys of the smoother parts of the area. These occur in that portion of each valley lying below the head water ravines and above the point where the drainage area is large enough and the valley low enough for the formation of a perennial stream. They are usually about a quarter mile wide, occasionally twice that width. The side slopes are, as a rule,

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gentle while the adjacent hills are rarely higher than 100 feet above the valley bottoms. The soil is not true alluvium but is silt and clay washed from the adjacent slopes. It is dark brown in color, is practically free from stone, is all in cultivation and is fertile. The valley floors are not level like alluvial valleys but slope gently from the bluffs to the stream channel.

The Gray Silt Loam.

Along the tops of the ridges in the areas not affected by the processes just described are often narrow strips of stone free silt to clay soil. It is gray in color and rests on a stony red clay subsoil. The change from the former to the latter is usually a sharp one. This is apparently the oldest, and therefore the most thoroughly leached soil in the region. Its character here, however, is essentially the same as it is in many other places in the Ozark region where it occupies the tops of the highest ridges. It often has a whitish, ashy appearance due to its silty character and low humus content. Its timber growth is chiefly scrubby black oak and black jack. On wettish places post oak grows while black jack grows on the dryer, better drained land. There is a tendency for the top layer of the subsoil, usually having a greater amount of chert than lower down, to become cemented into a hardpan layer. This is usually local, however, seemingly not occurring under any very large area. On account of this hardpan, the leached character of the soil and its low humus content it is not a highly productive soil. It needs humus badly. If this were supplied abundantly in the form of barnyard manure or legumes plowed under the soil would be greatly improved. It does not grow clover well. Cowpeas grow well however and can become, under proper care, the redeemer of this soil.

Alluvium.

The other soil of the region is the bottom land alluvium. This occurs along all the larger stream valleys. They are all rather narrow. The soil is brown to grayish silt and clay loam to gravelly loam.

Crops.

Owing to persistent advertisement during the eighties and early nineties a large amount of the stony uplands of this region were sold and considerable areas were cleared and planted to orchards, especially near the railway line. Hundreds of men with no experience whatever and with very little more money were induced to buy land and plant orchards. Many of them thought that all that was necessary was to plant the trees and then wait for the harvest. Such ideas were

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fatal. On any soil and in the most favored of climates such ideas do not produce results in fruit growing. On the soils of this region only one result could be expected. That has been abundantly realized. Hundreds of acres of dead and dying fruit trees in brush grown, barren, so-called orchards can be seen. Fences are down and in many places the material burned by woods fires; cottages and more ambitious houses are racked, unpainted and unoccupied. There is a general absence of an air of prosperity. This is not wholly due to the soil. It is largely due to the credulity of men whose training made them wholly unfit for doing the skilled work required of the successful fruit grower. Such men had to learn that young fruit trees as well as young corn must be cared for, cultivated, pruned and defended from insect enemies. That has been learned. Henceforward orchards will be planted in this region by men with some other equipment behind them than blind enthusiasm. Large areas of this land are not adapted to fruit culture, nor to any other crop that has to be cultivated. The land is too stony. Such portions of the area as cannot be profitably set to grass may be utilized in the production of timber.

In the extreme southeastern corner of the area colored for Howell soil on the map is a small area of another soil. At the time the map was colored this area had not been studied closely enough to warrant its differentiation from the Howell Soils. It is only the northern end of an area of soil that occurs over a considerable area in the adjacent part of northeastern Arkansas. In Missouri it occurs in Ripley and Butler counties in a small area in the vicinity of and south of Pander in the former county and in a narrow strip along the extreme eastern border of the Ozark hill region in the latter county. The soil is gray to brown in color and is practically free from flint fragments. The subsoil is brown to red in color, rarely greenish or gray and is likewise practically free from stones. Like most soils derived from limestone it is shallow and on many slopes the limestone rock is exposed. Occasional areas of practically bare limestone ledges of considerable size occur. They are called cedar glades.

This is a moderately fertile soil, is easily cultivated but inclined to wash rather badly when the humus has been burned out of it.

The topography is rolling, the greater part of the area however can be utilized either as tillable land or for pastures. It seems admirably adapted to Bermuda grass.

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THE OZARK PLATEAU SOILS.

Location.

The Ozark plateau as here defined occupies the western central part of the region. It occupies the greater part of the drainage basin of the Gasconade river and most of the Missouri part of the White river basin. As a whole it is a plateau with valleys. Taken as a whole its elevation is greater than that of either of the other main soil division areas. This, however, is not sufficient to be a factor of any importance whatever in the agriculture of the area. The average elevation of the uplands is about 1250 feet above sea level.

Geology.

It is underlaid chiefly by beds of limestone, the upper layers, the ones underlying the higher areas of country, having a moderately high percentage of flint. The deeper layers, those that are exposed only in the deeper valleys, have a much higher percentage of flint and weather therefore into a much more stony soil. Along part of the eastern boundary of the region there is a belt of sandstone which disintegrates into a sandy soil. It does not extend along the entire length of the belt, but occurs chiefly in Dent county.

Topography.

From the point of view of roughness of country due solely to the number and depth of valleys and ravines, this region consists of two rather different areas; a southern and a northern. The southern area is much rougher than the northern on account both of the number and of the depth of the valleys. The dividing line between the two areas is the Memphis line of the St. Louis and San Francisco Railway. The southern area is drained by White river and its tributaries, the northern one by the Gasconade and the Osage. In the southern area the stream system is a widely branching one in which the tributaries are arranged, with respect to the main trunk stream as well as to each other, like the branches of a tree are arranged with respect to the main body of the tree as well as to each other. The upland ridges between streams having such an arrangement are bound to be crooked and very irregular in width. When this is the arrangement of valleys and ravines in an area that is filled with them and one in which they are all deep, the roughness of the country is evident. The northern area on the other hand is drained, as a whole, by long streams running for a long distance parallel to each other. Their tributaries are rather short or else run more or less parallel to the main streams. The valleys also in the area, except those of the very largest streams, are not so deep as those of the southern area. Between the streams

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there are long relatively broad and smooth ridges widely different from the narrow crooked ridges of the southern area. They partake of the nature of long strips of plateau. They are especially characteristic of considerable portions of Texas, Dent and Laclede counties, but occur in only slightly less perfect development in the rest of this area. Narrow belts of very rough country lie along each side of the larger streams.

At the present time the area of Ozark plateau soils is covered with timber except where cleared for cultivation. There was timber on the bottom lands when the country was first visited by white man but until within the last half century the upland was either prairie or very open woods. The timber was not dense enough to shade the ground nor to obstruct long distance views to any great extent especially in the winter time. Grass covered the ground in summer with a growth as high as the back of a horse.

The bottom land timber is essentially the same as that on the bottom lands of the Springfield soil area. The upland growth is Black, Red, Post and White Oak, Black Jack and a sprinkling of Hickory. Most of these trees have their own special habitat. They do not all occur in the same localities. The long strips and areas of high plateau constitute the typical home of the post oak and the black jack. These trees occur however in other topographic positions occasionally, but always in places where the soil has somewhat the same character as that of the plateau. The Black oaks occur on the stony ridges in the areas of rough country, the Red Oak, on the moister areas and on the slightly better soils of the hilly areas, while white oak occurs on north slopes and sparingly mixed with the other timber elsewhere but chiefly in the rougher country.

The Soils in General.

There are four general groups of soils in the plateau area of the Ozark region. Three of them are limestone soils or at least lie on limestone rock while the fourth is a sandstone soil. The three limestone soils are all more or less stony, are silt or clay soils, have a gray, brown or yellowish color with a reddish clay subsoil, are low in organic matter and low in phosphorus. The different groups are separated from each other on the basis of topography, timber growth, kind of underlying rock, color and the percentage of loose stones in the soil. The main basis for the subdivision of the area into the groups on the map is that of topography and character of underlying rock.

Each of the groups of soil shown on the map has several types or kinds of soil. The groups only are shown on the map. They are designated as the Lebanon, Vienna and Salem groups.

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The Lebanon Soils.

Distribution.

This group of soils occupies the high ridges and plateaus of the area. It occurs along two main belts, one lying between the Gasconade and Osage drainage basins, the other between the Gasconade and the White river systems. Running northward from each of these belts are subordinate belts of greater or less width and length. They lie along the watersheds between the main tributaries of these streams. There are however no tongues or belts of corresponding length or width running southward from the main belts on account of the short distance to the main streams on this side. The southward flowing tributaries are in all cases short, the northward flowing tributaries are long. The northern belt is followed by the main line of the St. Louis and San Francisco railway while the other is followed by the Kansas City-Memphis line of the same railway system.

The Soils.

These soils are gray, silty, with a varying percentage of stones. They are the oldest soils of the Ozark region, have been subjected therefore to leaching and weathering for a greater length of time than any other group of soils in the plateau, have a paler color as a rule and are covered chiefly, where not cleared, with scrubby timber. The largest areas of smooth land in the plateau are in the Lebanon soil area. Its most persistent characteristics are its growth of scrub timber, its smoothness, its relatively small amount of stone and the height of the country on which it lies. The following types of soil are found in the area of this group:

Black-Jack Gravelly Silt Loam.
Post Oak Silt Loam.
Black Oak Stony Loam.
Post Oak Stony Clay Loam.

The Black-Jack Gravelly Silt Loam.

This is the most prominent type within the group and occupies the largest area of any of them. It occurs typically in Laclede county, occupying the greater part of the western half of the county. Lebanon stands in the center of this area. It extends southwestward into Webster but not in a large body. An area of it lies in southwestern Camden and northeastern Dallas and small areas exist in many other parts of the plateau region.

This type of soil occupies the slightly rolling lands of the Lebanon soil area rather than the smoothest portions. It lies within the smoother part of that region but does not occur in the very smoothest

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parts of this smooth area. It is rolling enough to furnish good natural drainage.

The soil is a gravelly gray silt loam becoming yellowish a few inches below the surface. The amount of red increases downward so that the subsoil is usually a deep red. The percentage of stone increases downward. The more hilly the land, the more stony it is as a rule. On the slopes of the shallow valleys where they are steep the soil becomes in places quite stony.

One of the more persistent characteristics of this soil is the yellow color of the top part of the subsoil. Whenever that is exposed in ditches or on the roots of upturned trees it is very noticeable. This passes downward to a red stony clay subsoil within from 6 inches to 5 feet. On the gently rolling uplands the amount of stone is not sufficient to interfere with cultivation. There is usually a small amount running from 4 inches to a foot in diameter. This is not sufficient, however, to make the expense of clearing it off prohibitive. The rest of the stony material is in the form of smallish gravel from two inches in diameter down. This is all angular material. None of it is waterworn material. It has been left in its present place by the decomposition of the limestone rocks in which it formerly occurred.

In localities where there is no gravel in the gray soil layer the top of the gravelly part of the subsoil is a rather sharp line. In some localities this is rather hard, due apparently to cementation by lime. This kind of hardpan seems to be local merely and not to extend under the whole area of this soil. The greater abundance of stony material in the subsoil than in the soil causes the former to seem harder, when being dug into, than it really is. Farmers encounter this in wells, ponds, postholes and elsewhere and call it hardpan. It seems to be cemented into a true impervious hardpan locally only, and in small areas.

Until within the last fifty years practically the whole of the area of this soil consisted of grass covered plains with isolated black jack trees or clumps of such trees. These older trees are still standing in many places on the uncleared land but the space between the large trees is no longer covered with grass. It is a thicket of young saplings, chiefly black jack and post oak.

There are no permanently flowing water courses within the area of this soil. It lies on a watershed so that all its drainage lines are small though a few of them drain large areas. These latter have no permanent stream along them owing to the porous character of the soil and the rock beneath it. The water soaks readily into the soil and thence into the rock and seeks an outlet to the larger streams by springs. A relatively small part of the rainfall reaches the main river

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by surface streams. The channels in all the valleys in this region are dry the greater part of the year, probably about eleven months of the twelve. Small pools lie in the channels in places a considerable part of the year but they are isolated from each other.

On the whole, probably about one-half of the area of this soil is now in cultivation, along the railways a somewhat larger proportion. The less stony and the smoother portions have been selected so far for cultivation. There are still uncultivated, however, considerable areas of a fair quality of this land. It is being cleared somewhat, but the rate at which new land is being put into cultivation, cannot be described as rapid. There is very little, if any, government land left in this soil area. Some of it, however, is still held in large tracts by non-resident owners.

This is not a soil of great fertility. It has, however, a good physical character, the country in which it occurs is relatively smooth, rendering it possible to handle it in large bodies and its supply of mineral fertility is moderately good. Its phosphorous supply is considerably below the standard for a rich soil, but is sufficient to produce fair crops until more can be supplied artificially. The greatest need of this soil is nitrogen and humus. The next greatest need is good thorough plowing and a general cleaning up of loose stones on the fields so that thorough cultivation can be done easily. For the present the growth of clover and cowpeas cannot be too strongly urged. More live stock farming will follow. The soil, while not rich, has good possibilities when handled with sufficient intelligence and capital. Like every other part of the Ozark region it is not the place for a man who has no capital to start with if he expects to do more than make a living.

Physical analyses of several samples from this soil gave 15 to 25 per cent of sand, 45 to 55 per cent of silt, and 10 to 15 per cent of clay. In the subsoil the per cent of clay ran up to 30 in one or two cases, though the other constituents did not vary so much.

The percentage of silt and clay is high making it a soil on which grass ought to thrive. The principal things needed for this are abundant humus and more nitrogen.

The crops now grown on it are chiefly wheat and corn. The yield of corn is about 25 bushels, and of wheat, where it is not fertilized, about 8 to 10 bushels.

The local water supply is from wells and ponds. Water is got in wells at varying depths, but a large supply cannot usually be obtained at a depth of less than one hundred feet.

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The Post Oak Silt.

This is the soil of the post oak flats of the region. While the black jack soils occur chiefly on the Osage-Gasconade watershed, these occur on the Gasconade-White river watershed, and on the ridges between the main tributaries of the Gasconade. The largest areas occur in Texas county on the broad ridges between the Gasconade river and Roubidoux creek, between Roubidoux creek and Big Piney, and on the ridge east of Big Piney. Some of these belts extend southward to the main ridge. It occurs also in small areas scattered over the whole area of Lebanon soils. Wherever a flat topped ridge is found, or a flat portion of a ridge, this soil occurs. A typical area lies in the vicinity of Fowler, Texas county, and another near Dykes. The latter extends in a long narrow belt northward from Dykes to the Gasconade river. Considerable areas occur in Wright, in Webster, southeastern Laclede and southern Pulaski counties.

The timber growth is almost entirely post oak. Over much of it the virgin growth has never been cut. There is usually only a relatively small amount of underbrush. A considerable proportion of the trees are large, reaching a diameter of 12 inches or more. The growth is too thick for grass, although it is not usually brushy. Where the original growth of timber has been cut off within the last 25 years, the land, unless it is cultivated, is now covered with a dense growth of post oak saplings.

There is very little tree growth of any other kind associated with become a tough sticky bluish clay. On the more rolling areas it is some black jack and black oak.

The soil is a silt usually almost free of stone. The percentage of silt is about 60 and that of clay 12 to 15. It is, therefore, a fine-grained soil. It is gray in color down to 6 to 10 inches, becoming yellowish or bluish or gray below. On the typical flats, if the stone-free silt layer is rather thick, there is a tendency for the subsoil to become a tough sticky bluish clay. On the more rolling areas it is yellowish to brownish.

At a depth varying from six inches, or less, to three feet or more, usually about 14 inches, a harder, more stony substratum is encountered. The hardness and amount of stone is variable. In other places it is softer, though usually it is much more difficult to bore into this than the silt above. It is often hard even when the stones are small in size and few in number. The finer material among the stones is a brownish to yellowish silt or clay mixed with spots of tough, hard gray clay or silt. These latter do not cut easily and come up on the augur as hard or tough lumps. Whenever the stony layer is struck the subsoil is stony in varying proportions thence downward. It is

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the ordinary residuary soil of the region. On the edges of the post oak flats where they break off into rougher land this stony layer is exposed because of the erosion of the overlying silt layer.

The top of the stony layer is sometimes cemented with iron or lime and thus converted into a hard pan. In such cases the soil layer is apt to be either too wet or too dry. It becomes thoroughly saturated during wet weather but dries out rapidly and cannot draw a sufficient amount of moisture from below to keep up its supply.

Probably not more than 30 to 40 per cent of this land is in cultivation. A considerable portion of it lies at a considerable distance from the railway and for this reason is not much in demand for farming. Of the land in the Ozark region sufficiently free of stone to be cultivated with fair satisfaction, the post oak flats, although nearer stone-free than any other, are considered the poorest. There is no crop that will grow well on them, timothy being about the best of the ordinary farm crops. Corn rarely produces, without fertilizer, more than 20 bushels and when the land becomes worn it is less. Clover does not grow well but cowpeas grow with considerable success. This is probably by far the most profitable crop that can be grown on this land. Where the stony layer beneath the silt is cemented or made relatively impervious to water this soil will be a difficult one to improve. The problem is a greater one than with many of the other soils where the main thing to do at first is to get the stone off. When it is got into a condition so that it can be well cultivated it can be improved. The post oak flats have the problem of a tighter subsoil. The growth of deep rooted plants like cowpeas and clovers will help and it is probable that the growth of these crops is the most feasible method of improvement available at present for these soils.

The Post Oak Glade Soil.

This is a shallow soil usually but little more than three feet down to the solid limestone and often much less. The surface is gray to black and the subsoil varies from yellowish, through brown, tints to deep red. Where it is stony the percentage of stone is large, the stones are white, hard, undecomposed and angular. The growth is scrubby post oak. It gradually changes into the black oak type as the solid rock becomes covered with a deeper layer of soil and the stones in the soil become more decomposed and more broken up. It occurs on hillsides where the limestone ledges outcrop abundantly. In wet weather it is apt to be too wet on account of the water being retained in the soil by the limestone bed beneath it. It dries out rapidly also. Where deep enough not to be affected by this it is a fairly good soil. A physical analysis of samples in Texas and Pulaski counties gave

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an average of 40 per cent of clay, 35 per cent of silt and 15 per cent of sand.

It does not occur anywhere in large areas. It has a wide distribution however, but always in small areas, often of less than an acre and from this up to several acres, never running into square mile areas. A local phase of this soil is found in the heads of wide open shallow hollows, on gentle slopes, and in other faint depressions occurring in many parts of the Lebanon region. The soil is gravelly or stone-free and black in color down to a depth of a foot or more. Below that depth it becomes gray, bluish, yellowish, brownish or reddish.

The surface of these basins is often covered with low mounds 2 to 4 feet high and 8 to 20 feet in diameter. The post oaks growing in such places are small and grow, usually, in groups. This soil is known by the farmers as black limestone land. It is a fertile soil but cannot stand prolonged droughts as a rule. A physical analysis of a single sample from Texas county gave 18 per cent of clay, 46 per cent of silt and 30 per cent of sand in the soil and 36 per cent of clay, 40 per cent of silt and 15 per cent of sand in the subsoil.

The Vienna Soils.

Distribution and Topography.

This is the last of the important groups of upland soils in the Ozark plateau. The soils range from very stony to stone-free soils and from gray to black in color.

This soil lies at a lower elevation than the Lebanon soils. The latter lie on the areas of the old plateaus that have not yet been cut into deep valleys by the creeks, while the Vienna soils lie on the high bench lands along the main rivers of the plateau region.

These benches do not lie at exactly the same level everywhere. The elevation is determined chiefly by the size of the stream and the position on the stream. As a rule they are lowest, or in other words lie farthest below the plateau on the Gasconade and Osage rivers and higher in the smaller streams, being highest at their heads.

Along all the rivers and creeks there are two strips of Vienna soils, one on each side of the present valley. The two strips are parts of one and the same thing separated into two parts by the valley. The width of the whole bench belt from one side across the valley to the other varies widely. On the Gasconade it varies from a few miles up to about 15. On the smaller streams it is narrower. Within the Osage river basin, however, it is wide, occupying the greater part of the country, especially on the south side of the basin, reducing the Lebanon plateau soil areas to small patches along the main water-

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shed. North of the latitude of Vienna, there are practically no areas of plateau soils. In this region, however, the Vienna soil benches have been extensively cut up by the many valleys of the existing drainage system so that while the total width and area of the Vienna benches is great the soils made by modern erosion within these benches occupy a large part of the areas. Nevertheless considerable areas of Vienna bench soils occur, especially in Miller and Maries counties.

Although the Vienna benches are remnants of areas that were at one time either level or not very strongly rolling, there is at the present time very little level land on them. A considerably smaller proportion of the area is level than that of the Lebanon area. It has been cut up by the existing streams into a hilly area, being more hilly on the outer and inner borders of each belt and smoother along its middle. On the inner border of each belt (each half of the old valley), the existing creek valleys and ravines are more numerous deeper and steeper while on the outer border there are the hills that bordered the old valley lying between it and the smoother Lebanon plateau areas. As a whole, therefore, the Vienna soils occur in a hilly to strongly rolling country.

Timber.

Like most of the Ozark upland, this region was either prairie or open grass-covered woods up to the middle of the nineteenth century. One small area in Texas county still bears witness in its name, Ellis Prairie, to its former condition. The whole of the region is, except where cleared for cultivation, at the present time covered with timber. The trees are mainly Black and Red Oaks; the former predominating. In fact this area might be called the Black Oak area of the Central Ozark region, just as the Lebanon area is the Black Jack Post Oak area.

There are a few small areas in the Vienna soils covered with Black Jacks and still fewer where Post Oaks predominate. The latter occur, however, as occasional trees in the Black Oak areas and White Oaks occur also. On the well-drained nearly stone-free soils with abundant lime, Walnut, Elm and other softwood trees occur.

The Soils in General.

As stated above the soils are stony to stone-free silt loams, the percentage of stones varying widely. On the basis of roughness or smoothness of the country each belt has three strips or subdivisions; an outer strip of hilly country lying next to the Lebanon soil areas, a middle belt that is smoother and an inner belt that is rougher lying alongside the belt of Reynolds soils to be described later. The latter strip is

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usually somewhat more stony than the others while the outer strip has a more shallow soil as a rule and a more scrubby growth of timber.

As a rule the Vienna soils within the drainage area of White river are more stony than north of it. In fact there is a gradual decrease in percentage of stones northward over the whole area, being fewer in the northern part of the area than elsewhere. The color of the soil is gray and of the subsoil red. The proportion of stones is about the same in both soil and subsoil except locally. The percentage varies from almost nothing in a few places to 80 or 90 per cent in the inner hilly strip of the belts. There are four main types of soil, they are the Black Oak gravelly, the Post Oak silt, the Black Jack gravelly and the Black Oak Stony soils.

The Post Oak Silt Soil.

The Post Oak Silt soil exists only on the flat areas. It is much like the Post Oak Flat soil of the Lebanon area. Like that also it is always underlain by a stony subsoil, the top part of which is sometimes cemented into a hardpan. The thickness of soil down to this stony layer varies, but is rarely more than 18 inches. These soils as a whole are usually considered stronger than the Post Oak flat soils of the Lebanon group, although they are more difficult to cultivate and a much larger proportion of the area is wholly unfit for cultivation on account of the large percentage of stone in the soil as well as the rough character of the country. This is generally true of all the soil types of the Vienna group when compared with the corresponding soil of the Lebanon group. The Vienna soils are more stony and occur on a rougher topography than the Lebanon soils yet where they are not too stony to cultivate they are considered more productive than the latter.

There are several things which may aid in producing this result. The rocks from which the Vienna soils are derived are somewhat purer, more crystalline limestones and possibly have a lower percentage of magnesia than those from which the Lebanon soils are derived. The Lebanon soils may not be wholly residuary also. The Lebanon soils are older and have been subjected to leaching for a longer period of time than have the Vienna soils. The Vienna soils occur in a more rolling country where soil creep on the slopes is more universal than on flat areas like those of the Lebanon soils. This tends to keep the subsoil more broken up and corrects what tendency there may be toward the formation of closely cemented layers or hardpans.

A much smaller proportion of these soils is under cultivation than of the Lebanon soil.

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The Black Oak Gravelly Soil.

This is essentially like the same type in the Lebanon group. It seems to have, as a whole, a deeper red color in the subsoil and supports a larger proportion of hickory trees and of hazel and sumac undergrowth than does the corresponding Lebanon type. The smoother, gravelly Black Oak land of the Vienna group is, in many parts of the Ozark region, called "Hickory Bench land."

The Black-Jack Gravelly Soil.

This soil has a limited area. The largest body of it occurs in Franklin township, in the Cobbs creek country of Laclede county. It is somewhat dryer, more gravelly and paler in color than the corresponding type in the Lebanon group. On that account it is not so good a soil for all crops as the latter.

The Black Oak Stony Soil.

This soil in the Vienna area does not differ essentially from the Reynolds soil, especially when the latter occurs on stony slopes.

Crops.

The crops that are actually grown on these soils do not differ greatly from those grown on the Lebanon soils though the land is better adapted to corn and clover than the latter. The Black Oak soils in the Ozark region, wherever they occur, are natural clover soils. It grows no better, however, on the Post Oak soils of the Vienna area than on the Post Oak flats of the Lebanon area. Cowpeas also grow equally as well on these soils as elsewhere.

The yield of all crops is low. This is due chiefly to a lack of nitrogen in the soil. The phosphorus content is low also but the factor that limits production at the present time is nitrogen. The percentage of vegetable matter is low and becoming lower. It was low to begin with for the same reason that it was low everywhere in the Ozark region. The open porous nature of the soil permits its rapid destruction under cultivation. The future is dependent upon more live stock, more forage crops, less grain farming, less cultivation, more vegetable matter plowed under. When the vegetable matter in the soil has been greatly increased then the addition of phosphorus in some form will still further increase the yield, especially of grain.

The average of several physical analyses of the Black Oak soils in the area of this group gave 12 per cent of clay, 60 per cent of silt and 20 per cent of sand. The sand in most of these cases probably consists of very finely broken chert rather than grains of quartz, though these sandstones occur in the formations.

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The Salem Soils.

Distribution.

This group of soils occupies an area rather than a belt, occurring on the eastern side of the central part of the Plateau region. They occur as a continuous body of soils only on top of the plateau.

The Rocks.

The formation underlying these soils and from which they were derived outcrops in a narrow belt along most of the large streams of the Plateau region and elsewhere but except the area colored for Salem soils on the soil map the outcrop is narrow and occurs on hillsides where the soil is incorporated with debris from overlying limestones, thus losing its character as a sandstone soil.

Soil.

The soils are stony sands, sandy clays and loams with varying proportions of sand and stone and clay. They are residuary soils derived from the disintegration of a sandstone. The rock varies somewhat in character but is usually a rather pure quartz sandstone cemented with iron and holding a relatively small percentage of clay. Over a large part of the area in which this rock has determined the character of the soil the sandstone has weathered completely and the products of its weathering have become more or less incorporated with the products of the weathering of the underlying limestone. The sandy soil in such cases becomes orgillaceous and stony, though it is never stony to excess.

Topography.

The topography of the area in which these soils occur is that of a plateau dissected by shallow valleys. The local relief is but little more than 100 feet over most of the area. The streams all flow northward and owing to the great distance that they must traverse before reaching a large stream like the Mississippi and to their winding course they are unable to cut deep into the country. The valley sides are sloping, rarely too steep for cultivation. The valley bottoms are narrow. There is very little flat upland. It is, in short, a plateau dissected with shallow valleys. There are practically no permanent streams in the area. The valleys have stream channels in them but they are dry except during excessive rains. The drainage channels are all of them the upper parts of streams and are all small. As soon as enough of the small drainage channels have united to form one large enough and deep enough to hold a perennial stream it has cut beneath the sandstone level into the underlying limestone and into another group of soils.

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There is very little of the area of the Salem soils that has too rough a topography for cultivation.

Timber.

Until half a century ago the whole of this area was a prairie but not wholly treeless. It was set with occasional large trees or in places was open woods. The timber was nowhere, except along the bottoms, too thick to hinder the growth of grass. At the present time every spot not in cultivation is covered with a growth of saplings and brush. On the sandier portions this growth is almost exclusively Black Jack. Where there is a moderate percentage of clay Post Oak replaces part of the Black Jack and on the stony portions where the subsoil is the stony clay derived from the underlying limestones, Black Oak becomes common. The original open woods growth was chiefly Black Jack.

The Soils in General.

The soil types in this region, unlike those in almost any other group of the Ozark region, depend wholly on variations in character of the rock from which they were derived, not on the topography, that being practically uniform over the whole region. The soils are sandy loams. The types varying from each other in the percentages of clay and sand and in the character of the subsoil. There are two main soils. In both the soil is derived from the decomposition of the sandstone. In one of them the subsoil also is derived from the same rock. In the other the soil is derived from the sandstone, while the subsoil is the stony clay of the underlying limestones. This latter type occurs around the northeastern and southern borders of the region and along some of the larger valleys. The other occurs in the interior of the area. The distribution of the different types of interior soils is not yet determined definitely. There is an apparent increase in sandy character southward and eastward. The soils are all gray to brownish or yellowish in color with a subsoil of somewhat deeper red color. The grayness of the soil is a function of the amount of leaching to which it has been subjected. It is everywhere low in organic matter and is inclined to wash where the slopes are only moderately steep. The subsoil is free from hardpan and has in most places sufficient clay to enable it to hold a fair quantity of water. The soil is light, has good tilth where well cared for and cultivates easily. The proportions of sand silt and clay from several samples taken at various places are as follows: Sand 25 to 40 per cent, silt 45 to 55 per cent, clay 8 to 20 per cent in the soil and sand 25 to 40 per cent, silt 35 to 45 per cent and clay 20 to 30 per cent in the

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subsoil. About thirty-five per cent of the area of these soils is in cultivation. The cultivated area extends along all the valleys spreading out over the adjacent slopes but not often extending far into the upland. There are some isolated areas of the highest uplands that are cultivated but the proportion to the total area is small. The cultivated area is the valley and valley slope area. The ridges are still in timber. Very little new land is being placed in cultivation. So far as agricultural improvement is concerned this area is at about a standstill or is gong backward. Probably as much or more land that was formerly cultivated is being turned out as there is of new land being put into cultivation. One reason for this is the natural thinness of the soil. Yet this is not the only reason. It is partly due to the fact that owing to the decline in iron mining the country has been decreasing in population. On account also of the iron mining and its attendant wood cutting and charcoal industry agriculture has never been considered the chief industry of the area. It has stood second in the minds of the people. The soil is thin but it is not hopeless. It is a soil that will respond quickly to good treatment. It cannot be made into a good soil in one season nor without capital and it will always be a somewhat expensive soil to handle on account of its somewhat leachy character. This, however, is not excessive. Its tendency to wash and the moderate amount of mineral fertility that it contains are factors that must be considered. The cheapness of the land and the low cost of living will compensate to a considerable degree for these things. Handled in rather large bodies with intelligence, industry and capital it can be made to produce paying crops. It will grow clover fairly well but probably the sandier soils need lime. Corn on the uplands does not yield more than 25 bushels, except where special conditions obtain and wheat yields 6 to 12 bushels. The quality is good, however, especially on the bottom lands. Very little clover is grown. Timothy is grown for hay but the yield is small. In fact the yield of all crops is low on upland soil that has been in cultivation longer than ten years. The limit of production is set by the deficiency of nitrogen, which can be returned to the land without money cost by the growth of clover and cowpeas.

THE OZARK CENTER SOILS.

General Character of the Area.

The Ozark center as here defined is not in the geographical center of the Ozark region. It is, however, the structural center of the region. It is the area cone or center around which are arranged with greater or less regularity the belts of soil that have been described

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already, especially those of the Ozark Border and around which curve those that make up the Ozark Center area itself. It is the part that was bowed up the highest when the bowing up of the rocks took place, though it is not now the highest area of the Ozark region. It has been worn off more therefore than any other part of the Ozark region, since it was originally the highest part but can no longer maintain that position. This has been brought about by wear from the top not by down bending or down sinking of this part of the region. It is this part of the Ozarks therefore where wearing has gone deepest and exposed the oldest rocks. This area therefore is differentiated from the others partly because it is made up of older rocks than any others and therefore of different rocks. It contains the very oldest rocks that are to be found at the surface of the ground anywhere in the Ozarks. In other parts of the region these very oldest rocks are hundreds or thousands of feet beneath the surface.

This area differs also from the others in topography. While it is not the highest area as a whole yet it has the highest point within the state. It contains the group of isolated rounded mountains known as the St. Francis mountains. It contains also a large area of very badly cut up country, and a very small proportion of high smooth plateau. The ridges and watersheds are narrow. It is an area of thoroughly dissected or cut up country. It also contains the only areas in the state of low basins surrounded by high country—a kind of intermontane basin. It also contains the only large areas of pine timber that are now standing or has ever grown up in Missouri since white man knew it. It was more completely covered by forest growth when first seen by white man, even outside the pine area, than the other areas of the Ozarks. Its timber, where it has not been cut in the last half century, is much larger than elsewhere and there is a much smaller proportion of young growth and brush. Its differences therefore are sufficient to differentiate it thoroughly from the rest of the Ozark region.

Since its differentiation is based on its physical characteristics rather than on its geographic position it follows that wherever the same series of events has taken place on the same kind of material we shall have similar regions developed—or subordinate centers. There is one such real or true subordinate center, true in every respect, lying in Camden and adjoining counties and a second area, or subordinate center of a lower degree, lying in Douglass and Ozark counties.

Topography.

As stated above this area, taken as a whole, is the roughest of the soil areas in the state. This is due chiefly to the great number and

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the depth of its valleys. In all the other areas containing a single soil group there is more or less smooth high country or flat-topped ridges or else of gently undulating land making up no small proportion of the whole. In the Ozark Center these are practically absent. In the greater part of this region practically the only level land is the alluvium which lies in narrow strips along the main streams. A level or smooth upland of more than a very few acres in extent is of rare occurrence. The only areas of relatively smooth country within the Ozark Center are the low basins that occur in the eastern part of the region. These have a rolling to slightly hilly topography surrounded by higher country.

Another division of the topography of the region is the area of rounded hills, some of them higher than the dissected plateau portion of the region and some lower. Occasionally they are alone, surrounded only by other phases of the topography and at other times they are assembled into groups. Each hill however is usually rather isolated, except at its base. A group of them does not form long, level topped or even topped ridges. The existing streams have not shaped these hills from the existing upland by erosion but they were fashioned by erosion of long vanished streams and lay buried under the present upland until the existing streams by their work uncovered them. Many others exist not yet uncovered. The tops only of some have been uncovered.

The three kinds of topography therefore are: (1) the thoroughly and rather deeply dissected plateau; (2) the lower undulating basins and (3) the region of rounded hills.

Timber.

The timber growth in the Ozark Center region consists of four dominant trees with the usual amount of brush and shrubs. The four dominant trees are Black, Red and White Oaks and Pine. There was originally more or less pine scattered over the whole area though it was more abundant in the southern part. Pine does not grow anywhere to the exclusion of all other trees and in most places it constitutes not more than half the total timber. It grows chiefly on the southerly slopes and on the ridge tops, rarely on the northerly slopes. The Black oak grows on the dry stony ridge tops and on the southerly slopes while the white and red oaks grow almost exclusively on the northerly slopes. The easterly and westerly slopes maintain a more or less even balance of tree growth among the several varieties. In the low land basins above referred to red oaks predominate with considerable Walnut, Elm, Hackberry and other trees which grow on fertile land. Post oak is not unknown but it occupies a very subordinate position in the whole Ozark Center.

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The Soils in General.

The differentiation of the Ozark Center into groups of soil types is based chiefly on the kind of rock underlying the area of each group. There are four groups, the Reynolds, St. Francois, Lamotte and Fredericktown. The soils of each group are underlaid by a different rock from that of any other group. The Reynolds group is underlaid by a Limestone which has a rather large proportion of flint. The St. Francois group is underlaid by granites, porphyries and other igneous or volcanic rocks. The LaMotte is underlaid by a sandstone and the Fredericktown group by a limestone rather pure and practically free from flint. Each group of soils occurs in a region with a topography more or less distinct from that of any other. With one exception this topographic distinction is a pronounced one.

The Reynolds Soils.

Distribution.

This group of soils covers by far the largest area of any of the Ozark Center groups. It covers practically all of Shannon, Reynolds, Carter and Camden counties; large areas in Iron, Wayne, Crawford, Washington and Madison counties and small areas in other counties. Its area is several times larger than the combined areas of all the other groups associated with it in the Ozark Center.

The Rocks.

It is underlaid by a limestone containing a great deal of flint or chert. At the top of the formation there is usually a sandstone also, but it is not usually thick enough to have a marked effect on the character of the soil. Immediately below the sandstone or in the upper part of the limestone there is a great deal of flint. It often occurs in beds of practically pure flint ten feet and more in thickness. Since flint does not disintegrate into soil but merely breaks up and remains in the soil as stone fragments, it furnishes a great deal of stone to the soil where it is, as in this region, abundant in the rocks. There are other beds of flint lower down in the formation and these increase the stony nature of the soil. The middle portion of this thick limestone making up these soils is nearly free of flint, however, so that wherever it outcrops and the soil of an area is derived from it without intermixture from above it is about free from stones. The limestone, aside from the flint occurring in it, is a rather pure, coarse grained and usually crystalline rock. It has a relatively small amount of clay impurity so that on decomposition a small amount of soil is formed. This makes the soil more stony by increasing the relative amount of stone in it.

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Topography.

This is a plateau thoroughly cut up with streams. As a whole it is the most intricately dissected of any of the soil areas in the Ozark region. It is almost one uninterrupted series of narrow branching valleys and sharp ridges. The occurrence of even small areas of smooth upland are so infrequent as to be practically negligible. The only areas large enough for even a small farm are in Crawford and Dent counties where the Reynolds soils are not typical on account of being mixed with sand and clay from formations that originally overlay this part of the Ozark center. The tillable area of this group of soils is small and will always remain so on account of the roughness of the topography. The tillable soils are chiefly in the river and creek bottoms.

This is the area of the state that should be devoted to the growth of timber. Its topography is too rough to make agriculture, except in the valleys, profitable. Its soil is too stony to be cheaply utilized for growing farm crops even if its topography was more favorable to agriculture. Its soil, however, is strong enough to support timber that has commercial value and the soil layer is thick enough to permit the growth of such timber. In other words the limit to agricultural development in the region is not determined by a lack of fertility in the soil but in the difficulty of utilizing it for growing farm crops. The timber has long served as the basis of a small manufacturing industry and with proper methods of conservation it could long continue to serve the same purpose.

Timber.

The tree growth is Black, Red and White Oak and Pine with a small amount of Post Oak, especially in Crawford and Dent counties. Pine grows or has grown in nearly all the counties of the main area but it was most abundant in Reynolds, Shannon and Carter counties. The main area extended from these counties over into most of the others. It never grew in any part of the Camden county sub-area of the Reynolds soils. Most of the pine has been cut.

White oak grows on the northerly slopes and most abundantly in Shannon, Reynolds, Crawford and Washington counties. A considerable part of this timber is still standing.

Black oak and Red oak are less valuable. They grow chiefly in the southern counties. Not a large part of this timber has been cut except locally. Large areas of it, however, are of little value except for local building material.

The Soil Types.

Sufficient has already been said about the rocks and topography to give a fair general idea of the soil conditions as a whole. They are all

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residuary except the alluvium and vary from stone free silts or sandy silts to extremely stony silts.

There are four different kinds of soils in the area sufficiently distinct to merit recognition. They are the Reynolds stony loam, the Morgan gravelly loam, the Crawford silt and the Reynolds loam. The first three are upland soils, the last is the bottom land alluvium.

The Reynolds Stony Loam.

This is the dominant soil type of the region. It occurs in all the hilly parts and is almost everywhere forest covered. The timber is Pine, Black, Red and White Oak; Pine does not grow on any of the other types. The soil is derived from the disintegration of a very cherty crystalline limestone. It is gray in color, down to a depth of about a foot, then gradually changes through yellow and brown to a red subsoil. The fine material of the soil is clay silt and sand in about the proportions of 8 to 10, 55 to 75 and 10 to 25 respectively. It is open and porous and warm. The percentage of stone varies from 25 to 90. The stone may exist as small fragments of small gravel size or as fragments of several hundred pounds in weight. The subsoil has a higher percentage of clay and lower percentage of sand, has a deeper color and about the same proportion of stones. There is rarely any suggestion of a hard pan. Its percentage of nitrogen and phosphoric acid is low and potash is high. The lime percentage varies, usually it is low, however.

On account of the roughness of the country in which this soil occurs very little of it has been put in cultivation. It is too steep to plow profitably in most places and can be utilized only for pasture. Wherever there is enough soil for plants to establish themselves and to furnish moisture clover grows luxuriantly. It is a natural clover soil except where it is too stony or too much exposed to the sun. The best places for the establishment of clover pastures are on the northwardly and eastwardly facing slopes. These slopes have no pines but are covered with Red and White oaks and occasional soft wood timbers. On these clover and orchard grass will grow as soon as the timber is cleared and blue grass will take hold when the soil has accumulated sufficient humus. This will rarely take place on the southwardly sloping hillsides. They are too much exposed to the direct rays of the August sun. The humus is soon burned out and the soil dries out rapidly. These slopes are adapted to the growth of pine timber better than anything else that has any value to man. Grain crops are not grown to any appreciable extent.

This soil can never support a prosperous agriculture. It can be utilized only in connection with some of the other types, particularly the Reynolds loam. It can supply supplemental pasture land and tim-

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ber land to the latter. The farmer who owns a good bottom land farm finds it convenient to own some of the stony hill land for pasture and for the growth of building material. Where other conditions are equal this combination gives the farmer a great advantage over the prairie farmer.

To sum up the whole matter in one sentence: The Reynolds Stony Loam is not an agricultural soil.

The Morgan Gravelly Loam.

This soil is derived from the middle beds of the great limestone formation of the Reynolds group of soils. This part of the formation is almost free from chert and consists of massive beds of crystalline limestone. When it disintegrates it forms a stone-free silt.

It does not occur in a large body anywhere within the area. Its chief occurrence is on the hillsides within the area of the Reynolds Stony Loam and in bodies of relatively few acres in a place. It is rarely entirely free from stone. They drift down onto it from higher areas of stony loam but are usually broken up into rather small fragments making a gravelly rather than stony soil. It occurs in larger bodies in Crawford and Washington counties than elsewhere, particularly in eastern and southeast Crawford and southwestern Washington counties. It occurs also in Morgan county and to a slight extent in St. Francois and Iron.

It is gray to brownish or reddish in color gradually changing downward to a reddish subsoil. The latter is usually deeper red in color than that of the stony loam. The fine material of the soil, in this case making up most of it, has a higher percentage of clay and a lower percentage of sand than the stony loam.

Its deficiencies are nitrogen and phosphoric acid. Its physical analysis shows a favorable physical character. The small percentage of stones does not hinder its cultivation seriously. On account of this, however, and its almost universal occurrence on hillsides it washes badly where cultivated in grain crops continuously, especially where the humus has been about all burned out of it.

While this soil, under proper management, makes a fairly good grain soil its tendency to wash reduces its value for that purpose. As a clover soil it is equal to the best part of the stony loam and on account of its greater proportion of fine material and cooler nature it is a better grass soil. Its occurrence in small areas or patches makes it an expensive soil to cultivate. When put into pasture land or clover meadow owned along with bottom land the combination is a good one. The gravelly loam makes excellent and cheap pasture or clover meadow while the bottom lands are excellent grain lands. Such a combination

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is especially adapted to stock farming. The region abounds in springs of good water also. It is not the place for the 40 acre farmer, however. It must be owned in bodies of considerable size and must be handled by farmers with sufficient capital behind them. There is no market in the region for such crops as a man must grow on a 40 acre farm in order to support a family. It is too far, in most localities, from the railway to favor the development of the dairy, fruit or poultry industries. It is especially adapted to sheep and hog farming and the growth of stock cattle. It can probably never become a region where cattle feeding on a large scale can be carried on because of the very limited area of corn land.

The Crawford Silt.

This is the soil of the flat uplands of this region wherever they occur. It occurs most abundantly in southern Crawford county, east of the Meramec river and its drainage area. The type occurs in Dent county also and in small strips along the tops of the ridges in nearly all the other counties.

It is a gray to yellowish sandy silt to clay silt graduating downward into a mottled to bluish clay or silty clay. There is a general absence of deep color in the soil or subsoil. The proportion of sand varies greatly. In some localities remnants of the sandstone underlying the salem soil still exist. In such places the soil is sandy. In other places the sand is very small in quantity and the soil becomes almost a blue clay in character. This occurs naturally only in the flattest places or in sags. The finer parts of the soil have come from originally higher rock or else is a remnant of a silt deposit that at one time covered the whole of the Ozark region, existing at the present time only on the highest flat portions like this area or that of the Lebanon silt loam area. Its lack of color is probably largely due to leaching. It is the oldest soil of the region and has been subjected to leaching for the greatest length of time. The leaching has affected not only the coloring matter but all the soluble constituents of the soil. We have no analyses of a typical sample of this soil but its probable origin, present conditions and crops indicate that the most important deficiency in its mineral constituents is lime. It is especially deficient in that and until that is supplied it cannot be built up rapidly. Clovers do not grow on it very well. Its other deficiencies most urgently needing correction are humus and nitrogen. These deficiencies can be supplied in the same way as recommended for other soils of similar deficiencies. Clovers will not grow here advantageously. Cowpeas will, however, and they should be one of the constant members of any system of rotation adopted for these soils. Lime, where it is deficient, will have to be carted onto the land. It cannot be grown.

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The timber growth on this soil is Post Oak, Black Oak and Black Jack with occasional Hickory and other hard wood trees. The trees are all small, not approaching the size of the trees on the other types of soil of the group. It is a scrub oak region.

The water supply problem in the area of this soil is different from that of the other types. There are no surface streams and no springs. Water must be obtained in wells and in reservoirs.

The crops grown are essentially the same as those grown on the other soils. The yields are very low, however. Fruit growing has received some attention and fruit trees do fairly well. No large area however has been set in trees.

Probably about 15 to 20 per cent of this soil is in cultivation. The advantages that it possesses over the other types already described is the smoothness of the country in which it occurs. Its disadvantages are its natural infertility, and its inconvenient water supply. It is not a hopeless soil but it is one that will cost rather heavily by the time it is put into good condition.

The Reynolds Loam.

This is the bottom land soil of the region. It occurs along all the river and larger creek valleys in strips of varying width rarely reaching more than three-eighths of a mile. The average width is considerably less than a quarter of a mile. The channels of the creeks change from place to place in the bottoms during floods. This reduces the width of bottom land available for cultivation considerably. In time these streams may be controlled but not in the near future. The areas are somewhat patchy also because of the wandering of the creek or river channel from one side of the valley to the other. The land is not perfectly level. There are low terraces in all the valleys and the soils included in this type extend up the more gentle neighboring slopes to a greater or less distance. The division line between the true alluvium and the residuary soils of the gravelly loam, is not sharp. True water laid alluvium extends up the slopes somewhat and above that is a zone of soil resulting from the wash of fine material from the higher slopes. All of the soil of this character is included in this type. In detailed mapping they would be separated.

The only timber left standing on the bottom lands consists of strips along the streams and covering the area that is overflowed by moderately high water.

This is either very low and rich land or else nothing but gravel bars. The trees are Sycamore, Elm, Hackberry, several kinds of Hickory, Red Oak and White Oak, Walnut, Burr Oak and Pin Oak. Cotton-wood rarely occurs, Linden occasionally, Birch very rarely and

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Black Gum rather common in the small, high, dry, very gravelly valleys.

The soil varies from a black stone-free loam to a light gray gravelly to stony clay loam. The true alluvium is usually stone-free and dark in color, though occasionally lighter colored to reddish. These, however, on the smaller creeks do not by any means occupy the whole width of the valley floor. Every tributary small hollow opening from the adjacent hills brings small stones, clay and silt during heavy rains and spreads them out on the main valley floor around where it opens into it in the form of an alluvial fan, built up higher at the mouth of the hollow and sloping off in all directions from this. This buries the alluvium of the main valley under this material. It may be quite stony or merely gravelly. It is rarely free from stone. The greater the proportion of stone, the higher it is built up at the mouth of the hollow and the steeper its slope down to the level of the main valley alluvium. If the hollows are small they do not have a channel across the bottom into the main creek but the water spreads out over this fan of material that it has deposited, sinks into it and gradually seeps through it and out onto and into the main bottom. This is apt to make a wettish strip in the main alluvium around the feather edge of the fan.

The main valley alluvium changes gradually downward to a gravelly and sandy clay and finally into a gravel bed or sand bed at depths varying from 5 to 20 feet. The fan of soil washed in by the small hollows continue uniform to the bottom of the fan material, below which comes the true alluvium of the main valley. The subsoils of the wash slopes gradually become either gravelly or more stony and pass into the unmodified red stony silts of the residuary soils of the region.

Occasionally there are terraces in the main valley with a gray stone free silt soil and a clay subsoil. This is a rather rare occurrence, however. Hardpan is of very rare occurrence in the alluvial soils. Occasionally the gravel layers may become cemented but such occurrences have not been met with.

The physical character of these soils is good. They are porous, warm, well-drained soils, not too stony to be easily cultivated and do not have enough clay to make them heavy. They dry out and warm up early in spring and promote rapid growth, both on account of their physical character and their protected position.

Their store of mineral fertility is greater than that of any of the upland soils. They are rarely badly deficient in lime, their potash content is high and their phosphorous content moderate. These minerals are being continually supplied to them by the seepage water from the adjacent hills.

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Their supply of humus when first cleared is good. Forest fires did not run through these bottoms every autumn like it ran over the hill lands. The leaves and grass were left to decay and supply humus. Under cultivation, this is rather rapidly burned out. On account of the more luxuriant growth of vegetation on this soil it is not so rapidly exhausted as on the upland. Considerable straw and other waste furnish a moderate supply. This is not sufficient, however, to keep it up to the standard requirement. The amount is being gradually exhausted and is having its effect in decreased yield. This is, in practically every case, the cause of the decrease. The wearing out of the land is not due to the serious reduction of its mineral fertility but to the exhaustion of its humus.

This soil does not wash badly. Some of the steeper slopes will wash but the proportion of land injured in that way is not great. It is not a leachy soil. The percentage of clay is sufficient to retain the fertility put onto the land in manures or in moderate amounts of commercial fertilizer.

This is a grain and clover soil. The grains chiefly grown are corn and wheat. The yields of corn vary from 25 to 60 bushels and of wheat from 10 to 25. Clover catches easily and produces a good yield though it is not as extensively cultivated as it should be.

This is the soil that produces the agricultural products of the Reynolds area. Practically all of it is in cultivation and has been in cultivation for many years. It is the seat of many good farms and prosperous farmers. On account of the limited area of this land it is held at as high a figure or possibly a slightly higher figure than land of the same productive capacity in places where the whole body of tillable land is good.

The owner of a body of this land, however, is the wealthy man and to a certain extent the leader or one of the leaders in his community.

The small amount of tillable land and the large amount of vacant land give cheap pasture, cheap fuel, cheap building material for most purposes and abundant room. Life conditions are somewhat primitive but not necessarily less attractive.

The Fredericktown Soils.

Distribution.

This group of soils occurs in a number of separate basins in the eastern part of the Reynolds soils area. The soils are all residuary and are derived from limestone beds that are practically free from flint. This yields a stone-free soil except where fragments of the limestone

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are found in it. It has no flint. There are two kinds of limestone underlying this soil. One is crystalline, massive, rather coarse-grained pure limestone or magnesium limestone. The other is a rather shaly, impure limestone containing considerable clay in its composition. The latter limestone is sometimes associated with thin beds of limy shale. Sandstone beds and beds of sandy limestone are of very rare occurrence.

Topography.

The topography of the areas in which these soils occur is a rolling to slightly hilly plain. There are basins also being surrounded by higher land on all sides. There is very little level land but there is also a relatively small amount of land that is too rough to cultivate.

Timber.

There is very little timber now standing on these soils. The most abundant tree is Red Oak. There is some White Oak, Post Oak, Elm, Walnut, Laurel Oak, Sycamore, Wild Cherry and other bottom land trees.

There is one large area and a great number of small, wholly or partially disconnected areas, in all about twenty in number. They are all alike in rock, soil, topography and relation to the surrounding country.

The Soil Types.

There are two kinds of upland soils in this region, both of which are probably groups of two or more types. There is also an alluvial type. The upland types are, (1) The Caledonia Brown Silt Loam, (2) the Bismarck Gray Silt Loam.

The Caledonia Brown Silt Loam.

This is a reddish, brownish, yellowish to grayish silt, to clayey silt soil free of flint with a deep red clay or silty clay subsoil. The thickness of the soil varies from a few inches to about a foot. There is no marked change or sharp boundary between soil and subsoil. There is a progressive increase in red color downward practically from the surface or from near the surface. There is no essential difference except in color, the clay content and the amount of humus. Analyses of several samples taken from widely separated places within these basins gave an average percentage of 23, 70 and 12 of clay, silt and sand respectively in the soil and of 30, 60, 10 per cent in the subsoil.

This soil occupies the center of each of the main basins. It occurs around Caledonia, at Belgrade, Farmington and southward to Libertyville, Fredericktown, Belleview, along the St. Francois river below Saco, around Patterson in Wayne County and on the Meramec river south of Sullivan. It is marked everywhere by its red color and by the growth of Walnut and Elm trees. It supports the growth of

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some fine Red and Laurel Oak timber and scattering White Oak. Its topography is usually slightly more rolling than that in which the Bismarck Gray Silt Loam occurs. The limestone beds outcrop adundantly along the creeks in these areas. The depth of the soil layer is not so thick as that of most other types in the Ozark region.

It is the most productive of the two types of soil in these basins. Its store of mineral fertility is fairly good; it is warm and easily cultivated; is well drained but holds a considerable amount of capillary water ready to be delivered to plants. It is a grain and clover soil, being somewhat better adapted to wheat than to corn though it grows both of them successfully. It grows clover luxuriantly.

The yields of wheat vary from 15 to 20 bushels and of corn from 20 to 50 bushels per acre. Where it has been run in grain continuously for years the former yields rule. Its decreased yields are due to the exhaustion of the organic matter, the nitrogen and to a partial exhaustion of phosphorus. Bone meal sown with the wheat usually gives marked increase in the yield. Clover in the rotation gives excellent results. Not enough attention is paid to growing it, however.

The only other upland soils in the Ozark region that rank with this in productive capacity are the Perryville and the better part of the Springfield soils.

Practically all this land is in cultivation and has been cultivated for a long time. These basins were the first upland soils to be extensively cultivated in Missouri. This is partly due to their occurrence in mining regions and partly due to their fertility. They were occupied next after the Mississippi river bottom lands. This soil stands in much the same relation to the agricultural wealth of the counties of the Eastern Ozarks as does the alluvial soils to the area of the Reynolds soil group.

The Bismarck Gray Silt Loam.

This soil occurs around the borders of the basins in which this and the preceding type occurs as well as in isolated areas here and there within the basins.

It is, like the Caledona soil, nearly free from stones, though on its outer border where it lies adjacent to a stony soil of another series it contains some stone. It is gray in color with a yellowish to reddish subsoil. The paler character of the soil and subsoil when compared with the Caledonia soil is due partly to leaching and partly to a slight difference in character of rock from which the two soils are derived. It has been stated above that the limestones of these basins present two varieties, (1) a crystalline, rather coarse-grained variety, (2) a finer-grained, somewhat earthy to shaly variety. The Caledonia soil is derived chiefly from the former and the Bismarck soil from the latter. The

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latter rock is the higher, geologically and occurs around the outer borders of the basins as a rule. Where the basins lie adjacent to an area of granite or porphyry, the gray character of the soil is due partly to the wash of soil from those areas.

When the limestone becomes shaly the soil is gray as elsewhere but the subsoil is much paler in color than elsewhere.

The surface of the areas where this soil usually occurs is usually undulating to slightly hilly, rarely rough enough to interfere with cultivation except locally. The timber growth is chiefly Red Oak with a small amount of white oak and some post oak on the flatter, partly leached areas.

This soil is not so productive as the Caledonia soil. It is easily cultivated however and where its humus supply is kept up it produces fair crops of corn and wheat. Clover does not do so well on it as on the Caledonia soil possibly because of the lack of lime and because of poor drainage. It is usually deficient in humus and therefore deficient also in nitrogen. It will produce fair crops of cowpeas on its thinnest parts.

It washes where it occurs on slopes but this is much more unusual than in the case of the Caledonia soil. The latter occurs along the drainage lines where it is more or less exposed to erosion and where its red color is, in fact, due to the continuous erosion from the surface of the top or leached portion of the soil. There is no sharp line separating the areas of these two kinds of soil. They graduate imperceptibly into each other.

The greater part of the area of the Bismarck soil is in cultivation, probably about 60%.

The Lamotte Soils.

General Distribution and Character.

This is a group of soils occurring in rather close association with the last two described. As a rule the country in which it occurs is a little higher than that in which those soils occur but is lower than that in which all other types that occur in its neighborhood occur. It lies adjacent to the areas of the Bismarck and Caledonia soils occupying therefore part of the basins in which they occur.

It is underlaid by a sandstone. It is the lowest and oldest sedimentary rock in the state. It occurs at the surface therefore only where the overlying rocks have been worn off. It lies immediately beneath the limestones from which the Caledonia and Bismarck soils have been derived. It may be coarse-grained producing a soil that is almost gravelly, though it is usually finer grained and often shaly. There is in all parts of it enough sand to make a soil that is quite sandy.

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It occurs chiefly in Iron, St. Francois, Ste. Genevieve and Madison counties. In St. Francois county it occupies a large area lying mainly northeast of Farmington. The eastern part of this area lies in Ste. Genevieve county. The Iron county areas are rather small and lie in the eastern part of the county. The Madison county area is larger and extends northwestward from Fredericktown, into St. Francois county as a belt several miles in width. Elsewhere this sandstone occurs in such small areas that they cannot be well shown on the map.

Around that border of these areas where the sandstone goes beneath the adjacent limestone the soil of the sandstone area is considerably modified by the remnants of the limestone soil still mixed with it. The St. Francois-Ste. Genevieve county area is surrounded on all sides with the limestone. Elsewhere the limestone occurs only on one side while granite and porphyry lies on the other.

Timber.

The timber growth on this soil is, practically everywhere on the uplands, a scrub oak. In a few places where the amount of clay in the soil is unusually great, White and Red Oak are found. The scrub oak consists of scrubby growth of Post Oak chiefly with Black Oak and Black Jack less abundant. The usual bottom land timber of the region grows on the alluvial lands of this area.

The Soils.

This area has not been differentiated into types. The soils vary from place to place in the percentages of sand and clay in them but not enough is known about the area to differentiate the soil into types. The alluvial soils are of course distinct from the upland soils but there are no large streams anywhere within the areas of this group so they occur in such small quantity that they may safely be neglected.

The upland soils are everywhere sandy but in few if any places are they too sandy for good agricultural soils. The percentage of clay is usually large enough to render them sufficiently retentive of moisture and to prevent undue leaching.

It is a stone-free soil and where the topography is rolling is somewhat subject to washing. It does not wash so badly as the Hillsboro soils, however, on account of its occurrence in a smoother area of country.

It does not produce heavy crops, partly on account of its natural barrenness but chiefly on account of the treatment given it.

Like all the soils of the Ozark region, it has good pysical character. Its mineral plant food is low except potash which is moderately high. Its lime and phosphorus are low. The main deficiencies, however,

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are its low humus and nitrogen content. Both these absolute necessities have been largely used up and are not being put back.

The neighborhood of the mining industry of St. Francois county has caused it to be neglected to a still greater extent during the last ten years than formerly. There are some abandoned farms on it.

This soil is not so good as either of the soils in the preceding groups. Its area stands in rather sharp contrast with those areas, both in the proportion of it in cultivation and in the character of the improvements found on the farms. These features stand out clearly in a general view of the areas of both kinds of soil, such as can be obtained from high points on the hills surrounding the basins in which they occur. Not more than 50 per cent of the LaMotte area is in cultivation while fully 75 per cent of the surrounding area of Caledonia soils is cleared.

The St. Francois Soils.

This small group of soils covers an area having the least agricultural value of any of the soil groups in the state. An extremely small proportion of the area is in cultivation. This is due partly to the nature of the soil itself and partly to the topography. These soils occur in small areas scattered over several counties in the eastern part of the Ozark region. The largest areas are in Iron, Madison, St. Francois, and Washington counties. Smaller areas occur in several other counties.

The rocks are granites and porphyries. These are hard, usually compound rocks of igneous origin. They disintegrate very slowly usually, especially the porphyry. These are the lowest rocks that are exposed at the surface within the boundaries of the state. They underlie all the other rocks and so far as our knowledge goes there is no rock of a different character underlying them. They seem to extend downward indefinitely. They make up the foundation on which the rest of the state was built. It is only in a few places where these rocks have been bowed up that they are exposed at the surface by the erosion away of the rocks that belong above them.

These rocks have been encountered in deep drill holes in several parts of the state, at Carthage at a depth of nearly 2000 feet, at St. Louis at a depth of about 3000 feet, in Camden county at a depth of about 1200 feet.

The topography is rough. Only a very small area of it is smooth enough to cultivate. The greater part of it consists of steep mountain sides Almost all of the small areas shown on the map is a hill from 200 to 700 feet higher than the surrounding country with rounded peak-like top and steep slopes. The larger areas consist of groups of such hills with narrow deep valleys between. The

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valleys are mere gorges, usually too narrow for even small fields. The bare rock is exposed frequently on these steep hill sides, though there is enough soil to permit the growth of a cover of trees.

The only level tracts are two or three rather small areas underlain by granite. One area lies northeast of Ironton. It extends northeastward to the vicinity of Knoblick.

The timber consists of Black Oak, Red Oak, White Oak, Post Oak, and Pine. Black and Red Oak are the most abundant trees. Post Oak grows on the few areas of flats. Most of the timber now growing on these areas, especially in St. Francois, Madison and Washington counties, is second growth. The original virgin forest was cut for charcoal during the time when iron smelting was an important industry in southeastern Missouri.

There are two general soil types, neither of which, however, is of any importance in agriculture.

One is the stony hillside soil of the porphyry areas. The fine material is a gray silt and clay with a yellowish gray subsoil. It is usually full of angular fragments of the rock, varying in size from small sand-like grains up to tons in weight. They would seriously interfere with cultivation even if the land was smooth enough. Of the total area of this kind of country in the region, amounting to several hundred square miles, probably not one square mile is in cultivation.

The other soil is the gray silty clay overlying the more level granite areas. This is a silt or silty clay gray in color with a yellowish subsoil. The amount of stone fragments in it is, as a rule, not great. In places it is nearly free of stone. It is a rather intractable soil not easily cultivated and yielding very little crop.

Probably one-fifth of the area of this soil has been cleared and cultivated but it cannot be called an agricultural soil.

The area including the St. Francois, LaMotte and Fredericktown soils is, from the point of view of scenery, one of the most interesting in the state. This lies in the great contrasts between the level or gently undulating areas of limestone soils in the limestone basins, well cultivated and dotted with prosperous looking homesteads, and the steep, high, timber covered mountain sides surrounding them. The smooth areas run up to the foot of the mountains and the change is a sudden one.

Another feature lending interest to the country is the contrast, along the same streams, between the low grass-covered banks and the broad valley in the limestone basins along which the streams flow in a leisurely way, and the steep-sided, deep, narrow valleys or the "shut ins" where the streams plunge noisily over the stones in their narrow channels.

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PART II.

AGRICULTURAL CONDITIONS IN THE OZARK REGION.

THE REGION IN GENERAL.

The Ozark region of Missouri is unique. It is the only region of its kind in the world. Its combination of special characteristics differentiate it not only from its surrounding country, but from all other known regions of the world. Its special characteristics are the product of the four factors of character of rock, geological structure or attitude of rock, erosion and climate. The first of these factors is the fundamental factor. It is the one that is unique. It is the one that most completely differentiates the region from all others. The attitude of the rocks is not essentially different from that of many other regions. Erosion has acted in the same way here as it would have acted in any other region having the same kind of rock, while the climate does not differ from that of the country immediately east and west of it.

It is a region that, in comparison with the country north, east and west of it, has been somewhat unattractive as an agricultural region. It has lagged behind therefore in agricultural development. This has been due to the nature of the soil, the topography and the vegetation.

As shown in the first part of this report it is a region of stony soils, of rather hilly topography and of timber covered or brush covered land. In addition to these characteristics the soil, while not infertile, is less fertile than the black prairie soils of adjacent regions.

These characteristics served to retard its development as long as there was an abundance of fertile prairie land that could be had for the asking. It could not appeal strongly to the practical business like, far-seeing pioneer from the northern and northeastern states who came west in search of rich land that could be cultivated easily and quickly. It did not appeal to the man who desired to farm on a large scale and in the old way. It did not appeal to a grain growing farmer who desired to operate on a large scale. The timber and brush cover made its subjection to the plow slow and costly in time and money. It could appeal strongly only to a people who had adapted themselves to a timber covered, hilly country of moderate fertility.

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The Early Immigrants.

The people of the southern Appalachian region found in this region a nearer approach to the one to which they had adjusted themselves than any other group of migrating people in the country. The first settlers in the region were from this latter part of the United States. They were the only occupants of the region up to the latter part of the nineteenth century when railway building in the region brought in people from the north and from Europe. These latter, however, settled only along the railway lines. They did not spread out over the country as a whole. Even up to the present time the greater part of the region is occupied almost extensively by the descendants of these early settlers. The iron mining in the Central part of the region and the lead and zinc mining in the southwestern part attracted laborers from the outside but they rarely became part of the agricultural population. As long as the mining continued in any particular camp they continued with it and when it declined they left it. They did not take up land and become permanent settlers.

In the lead mining regions of the eastern and northeastern Ozarks, however, the mining was carried on, until recently, chiefly by the farmers. They mined in the winter months and farmed during the summer. They constituted part of the permanent population. In the eastern Ozarks, however, a considerable portion of them were of French origin, descendants from the early French settlers along the Mississippi river. They never extended west of the mining region and the number who became permanent Ozark settlers was small.

The best land of the region lies in long narrow strips along the creeks and rivers. Between these lie broader belts of rough, stony, timbered land varying from one to several miles in width. The people were, and in portions of the region are still, situated only along these valleys. Each valley is isolated, more or less, from the others. The people in each gradually develop characteristics peculiar to it or a certain style of dwellings, barns, crops or methods of cultivation. Whole valleys have become the property of one family; the original settler and his immediate family selecting only the richest of the alluvial lands. The second and third generations have extended the clearing to the more gentle and less stony of the adjacent slopes or even into the "flat woods" or "Barrens," the flattish undissected strip of upland along the axis of the belt between adjacent creeks. The development of the region as a whole, except a few localities near modern railroad towns where the presence of the real estate agent has caused a temporary boom, has been brought about in this way. It is the result of the natural growth of the native population. The influx from the outside has been small, except where the region has

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been occupied by Germans. In such cases the original population was bought out and the German population took the place of the original. The Germans were not *added to* the original population and there has been slight influx of Germans since the first colonization. The increase in German population has been the natural increase of the original population of the colonies.

History of Settlement.

The first settlement of the regon took place before the beginning of railway development in the United States. The first settlers were French colonists who came up the river from New Orleans. The permanent settlers remained near the river, while the miners and adventurers sought the lead region of what is now Madison, St. Francois, and Washington counties. French colonists settled at Ste. Genevieve in the first years of the eighteenth century, at New Madrid a few years later and St. Louis in 1765. The fact that the French settlers came in colonies and settled in villages together with the fact that the peculiar long, narrow subdivisions of land characteristic of the subdivisions of European village communities is found only at New Madrid and Ste. Genevieve indicates that the French did not do a great deal toward the permanent agricultural development of the Ozarks. All the chert-free limestone basins in the Eastern Ozarks are covered with French and Spanish grants but none of them have the characteristic long, narrow subdivisions. They are usually large tracts that were granted chiefly for mining purposes or else were unable to attract colonists. There are, however, many small grants that were made to permanent settlers but most of them seem to have been made shortly before the American regime commenced and after the influx of American settlers. Many of the names of the grantees are American names. This is not true, however, of many of the older and larger grants.

Practically no settlement took place in the region except that along the Mississippi and in the limestone basins of the Eastern Ozarks until near the end of the first quarter of the nineteenth century. In 1819 when Schoolcraft traveled across what is now Washington, Crawford, Dent, Texas, Wright, Douglas, Taney, and Ozark counties he found only one or two squatters, engaged in hunting chiefly, after he left the settlements at Potosi in Washington county. Probably occasional squatters had located themselves at various points in the valleys of the northern border but the great central and southwestern parts of the region were the winter hunting grounds of the Osage Indians. There were no roadways across the region at that time.

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The Early Roadways.

Probably no road extended further west of the Mississippi than Potosi earlier than 1820.

From Potosi southwestward, ran an ancient Indian thoroughfare—a mere footpath or bridle path. It was known by the white settlers as the Osage trace or the White river trace. It ran from Potosi southwestward, following approximately the line of the existing road from that place to Webster but passing about a mile north of where that town now stands. It ran thence southwestward to Huzza creek not far from where the village of Davisville is now situated. It turned thence southward for several miles and then again southwestward. It passed about five miles north of where Salem now stands, then across the head waters of Dry Fork of Meramec to the vicinity of Licking. Thence it crossed Big Piney at Plattner's Dam, passed a little way to the south of Success and crossed Roubidaux Creek either at Turley or Flat Rock. It crossed the Gasconade river at Hartville and ran thence to where Springfield now stands, probably running near the present site of Marshfield. From Springfield it followed the track that was later called the "wire" road, running from Springfield to Forth Smith on the Arkansas river. This latter end was not a part of the Osage or White river trace.

This became the great settlers thoroughfare for the region, at least for the southern and the southwestern parts. They came in two streams, both originating from the same region—the hilly region of the Southern Appalachians. One came across Tennessee and Kentucky to "The Iron Banks" ferry, where Columbus, Kentucky, stands at present. Their route lay thence northwestward across the lowlands through Charleston and Benton to Jackson. Then it followed the old Jackson and Farmington road to the latter town where it met the other stream which had come across Kentucky and Southern Illinois to Ste. Genevieve or Herculanaeum. The united stream flowed thence southwestward along the White river trace through Caledonia, Courtois Diggings, The Lick Settlement (Licking) and Hartville to Springfield. Here the White River trace joined the St. Louis and Fort Gibson road running southwest of Springfield across what is now Greene, Christian, Stone, and Barry counties into Arkansas. Before 1840, however, many roads had been located in all directions across the region.

The Early Settlers.

The settlers who came to this region were not the poor whites of the slave-holding regions of the south—the lower coastal plain and the Piedmont. They were the more independent hill people from the more mountainous sections where they had had rather remote

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relations with the slave-holding class. Occasionally an especially prosperous man among these owned a few slaves which he brought with him but they were few. They came from a hilly region where tillable land consisted chiefly of the alluvial strips along the streams, where the streams were clear, springs abundant and timber plentiful. They sought similar conditions in their new home. These they easily found. There was one rather important difference, however, between the country they left and the one to which they came. The one they left was heavily timbered, both hills and valleys. The greater part of the one to which they came had heavy timber in the valleys and on the very stony soils while on the smooth upland between the streams there were large areas of park-like, open grass land. The few trees were large as a rule. Brush patches or areas of small timber rarely occurred. In the Ozark Center region flat upland watersheds were rare in their occurrence so this area was a forested region. The virgin timber standing on it at the present time shows that it has been timber covered for at least two to three hundred years. On the Ozark Plateau, however, there are large areas of smooth upland. These were treeless prairies or grassy oak openings until the middle of the nineteenth century. The same statement can be made concerning all the western Ozark Border.

The Early Agriculture.

The prairies and oak openings furnished abundant summer pasture for large herds of cattle, and where the dry grass was not burned early in the winter it fell over making a natural blanket over the whole country beneath which the grass remained green until late in the winter. Old settlers assert that young stock would go through the winter without other feed than what they got from the range. Much of the winter pasture, doubtless lay in the more protected bottom lands. The timber on the bottom lands and elsewhere was sufficient to furnish abundant mast not only to keep hogs alive but to prepare them for market. The virgin lands of the creek and river bottoms produced good crops of corn also with a comparatively small amount of labor. The conditions were extremely favorable, therefore, for stock raising. There was abundant and cheap food and abundant water along all the rivers and large creeks and at the many springs which occurred in almost every hollow.

Grain farming for the market was impossible on account of the lack of facilities for transportation. There were no railways. The wagon roads were rough and hilly. The streams were, except in rare cases, not navigable. Lumbering also was not possible because of the same lack of facilities for transportation and also the lack of

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a market for such lumber as the region would produce. The mining industry until the middle of the century was confined to a few counties in the eastern part of the region. It has never been of any importance in the south-central part of the state and has fluctuated even in the mining regions greatly within short periods of time. The only two parts of the region where it has been in any way permanent are the eastern and the southwestern. The natural conditions surrounding the original settlers, therefore, made stock raising the only industry that was practicable. Stock could easily be driven to the Missouri, Mississippi, Lower White and Black river shipping points.

The Second Agricultural Stage.

So long as cultivation was confined to the alluvial strips along the creeks the products of the soil were sufficient to satisfy all demands of the people. The bottom lands furnished an abundant supply of grain for all purposes while the uplands furnished abundant free range for everyone's live stock. The original immigrants did not occupy all the alluvial land. There was in most cases a supply for the first generation of descendants. By the time the third generation were ready to become farmers, however, the bottom lands were all in cultivation. They had to make farms from the less desirable lands of the uplands or the hills. At first they selected the level lands of the axial belt of the dividing ridges. These were smooth and, in most parts of the region, free or nearly free from stone. The soil is not fertile—not so productive as the stony lands of the hilly belts adjacent to the valleys—but they were much easier to cultivate and were usually free or nearly free of timber. They were easily put into cultivation.

This invasion of the upland effected a profound change in the economic life of the people. It did not take place suddenly but came on gradually, almost imperceptibly. In many parts of the region, particularly the central part, the readjustment in response to it has not yet taken place. The fencing of part of the upland reduced the free open common pasture land and therefore the food supply for live stock to that extent. Another change took place, however, that had a much greater effect in decreasing the available grazing land than the fencing of part of it. Even at the present time there is still a large amount of land not under fence.

The Consequences.

As a result of the building of rail fences on the ridges and partly also those in the valleys the annual prairie and woods fires were gradually stopped. The fencing material was inflammable and had to be protected. Fires were stopped, therefore, wherever possible or at least

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much restricted in their original freedom. The stopping of the woods fires allowed the brush to spring up and grow. As long as there was an annual fire which raged through grass from three to eight feet high and thick on the ground, all forest-tree plants were killed down to the ground every year. During the ages preceding man's appearance in the region acorns had been scattered well over the region. Young plants sprang up from them every spring but were killed down in the fall. The roots were not killed, however, but remained to send up young shoots every spring. They in turn were killed in the succeeding autumn. This continued for ages. The roots remained alive and spread through the ground, making underground rootstock networks often nearly filling the ground down to a depth of a few inches. When the annual fires were stopped these roots sent up hundreds of shoots which grew rapidly and in the space of a few years produced a mass of scrubby brush that smothered out the grass completely. At first the brush was so thick it could not grow to any size. For many years it stood at about the height of a man's shoulder and from that height down. Gradually the more vigorous individuals got a little ahead and the weak ones died out. At the present time there are small areas scattered abundantly over the nearly level high silt or clay ridges of the Ozarks covered with a thick growth of young Post Oak or Black Jack trees from 2 to six inches in diameter that have grown from such brush patches. All these ridges are now covered with such a growth where they are not in cultivation. The result is that the grass has been completely killed out. In many parts of the region there is abundant vacant land to furnish range for more stock than the country now has but it is covered with young trees rather than grass.

In the hilly, stony belts of country between the smooth clay ridges or the original slightly stony ridges and prairies, the same process has gone on at least so far as the result is concerned. The growth of timber has completely killed out the grass in some places and greatly injured it everywhere. It has not been affected by the growth of post oaks, however. The hill lands are not the natural habitat of these trees. They occur only sparingly. The trees that grow on the stony lands are Black, Red and White Oaks. None of them will form underground stools like those formed by the Black Jacks and Post Oaks of the flat clay ridges. There were enough large trees, however, to keep the ground well seeded. As soon as the fires were stopped young trees started up from these seed, making a cover much less dense than the post oak and black jack "runners" of the smooth uplands but dense enough to smother out the grass.

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At the present time there are relatively few areas of any size where the original Blue Stem grass, which grew everywhere over the region originally, grows with even half its original luxuriance. There is one area lying in Morgan and Camden counties, another in the Pine region of the southeastern Ozarks, and a third in parts of Ozark and Taney counties. In the rest of the region small patches occur but the great areas of woodland produce as a rule nothing but weeds or chiefly weeds with a small amount of grass. The range, however, in much of this stony, hilly belt even where most of the grass has been killed, is still much better than are the timber belts of the flatter ridges. The grass is somewhat more abundant, but in many places the ground is covered by a dense growth of annuals, many of which belong to the Leguminoseae. In the autumn large areas of wild pea vines and "Beggar Lice" furnish excellent grazing for cattle.

It is possible that the growth of trees and brush has not been the sole cause of the killing out of the grass. It may have been caused partly by overgrazing and tramping. The effect of the latter, if it was effective at all, was probably confined to the clay lands of the ridges. The stony lands could not be injured by the tramping of cattle and horses. The fact that the range continued to be good until the brush covered the country is good evidence that the shade and moisture consumption by the trees were the principal factors.

The Abandonment of Stock Raising.

The destruction of the natural pastures has caused the abandonment or serious curtailment of the stock raising industry. The making of artificial pastures of tame grasses on the cleared lands has not kept pace with the destruction of the natural ones. That is evident, since the destruction of the original as pointed out above is the result, not of the taking up of the land and putting it into cultivation but of the growth of brush. The making of the tame grass pastures is a development of the future. The old Blue Stem was naturally adapted to the soil and climate. It had become so through long ages during which the law of the survival of the toughest, the strongest, the best, was the only law that prevailed. Man has not yet found or at least has not universally adopted a substitute for it.

The Adoption of Grain Farming.

The change described above has come for the whole region. It came in some parts of it earlier than in others, depending chiefly upon the rapidity with which the upland was occupied and the native grasses killed out. It cannot be described, however, as a change peculiar to the Ozark region. It is one that has taken place over

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a large part of the United States. The earliest settlers were primitive stockmen, growing their stock on the native grasses of the unoccupied land. The change did not come in the Ozark region until long after it had taken place in the greater part of the area surrounding it because of the slower rate at which the former was settled up. The usual farming system, following the primitive stock raising, is a system of grain growing. This takes place in practically all cases where the soil is fertile, the land smooth or relatively so and where means of transportation exist. The Ozark farmer followed the same order of economic development. He became a grain grower and ceased to be a stockman or at least decreased greatly his operations as a stockman. This change took place in the Ozark Plateau and Ozark Center within the last 15 or twenty years. It took place in the Ozark Border from 20 to 35 years ago or even earlier than that in a few cases.

The Results in the Ozark Center and Ozark Plateau.

In the Ozark Center and Ozark Plateau, taking these areas as a whole, the income from exclusive or nearly exclusive grain growing has been small. Grain farming has not been profitable. The upland soils do not produce profitable crops of grain for many years in succession by the ordinary method of cultivation. The bottom land is too limited in amount to produce sufficient grain to raise the income of the country as a whole up to what it was when the total land area was producing something. The change has not been a satisfactory one. It came with the force of a demand and without invitation, though as a result of the action of the people of the country. The decreased income of the individual has resulted in unrest and dissatisfaction. The people realize that the country cannot be prosperous as a grain growing region but they do not yet, in the region as a whole, see what to do next. They realize that a new adjustment must come but have not universally agreed on the particular kind of adjustment that is necessary. Sporadic attempts have been made to find the proper remedy. Many men have left the country to find free range and all its attendant attractions to the born and bred pioneer in the far west. Others, more willing to change their mode of life, have been attracted by the alluring literature of the fruit-tree nurseryman and the railway advertisements. Still others and much the larger number have merely curtailed their operations—are growing fewer cattle and hogs and therefore making less profit. The improvement of the quality of the cattle, sheep and horses has not been sufficient to compensate for this curtailment. Hogs, however, have been improved so that as good a grade of hogs may be found in the Ozarks as in most parts of the state. The average farmer at the present time has a smaller net income than he or his progenitor had fifty years ago. This is true of the

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bottom land farmers as well as the ridge farmers. The farms that were orignally large have been subdivided until they would yield to each head of a family a smaller total income even if he had access to free range as he formerly did. This also has been destroyed so that his income has been still further diminished.

Social conditions in parts of the region have not improved and and in some ways they are not so pleasant to the existing population as they were under the old regime. One of the factors, and an important one, producing this result is the constant drain made on the most vigorous and effective part of the populations of the country. The young men and women and many of the more progressive and socially desirable middle aged men and women leave the region for the cities or for the attractively and skilfully advertised lands of the west.

Another factor is the change which has taken place in the facilities for communication. Formerly the roadways were the sole transportation lines. All products consumed by the population except those grown or produced at home were transported long distances by wagon. All products taken out of the country went by the same routes. They were kept well beaten out, therefore. They were laid out with the demand of this traffic in mind. There was practically no other factor controlling their location. This caused their location along lines of easiest passage—along the valleys. Usually they ran along one side of the valley, being confined to this by the farms. They wound picturesquely along these valleys through the walnut groves by the farm houses, never in straight lines and also rarely over the hills, except when necessary to pass from one valley to another.

The present traffic is less than formerly. The roads are, therefore raw, gullied, stony, untamed and usually unworked. They have been shifted from their former courses which were determined by the route offering the least resistance to one that follows the land lines irrespective of the shape of the country. They run, therefore, in straight lines across deep hollows, up steep hills, wholly disregarding the nature of the country. There has been very little attempt to grade them and no attempt at proper drainage. On the slopes therefore, the road is soon worn into a hollow, down which the drainage water flows during heavy rains. The finer materials of the road are soon washed out, leaving the surface covered with a layer of loose stones of varying sizes up to several inches in diameter. In addition to this it is often badly gullied. The stones and gullies make the hauling of a load over it impossible and any kind of passage over it difficult.

Since the advent of the railway in the region the roadways are not used so much for hauling heavy loads but their disuse and lack of care results in a serious drawback to the social life of the people.

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The conditions of life within the region at the present time vary greatly with the character of the country. In no other part of the state is the contrast between the income of the owner of poor land and that of the owner of good land so great. The difference between the productive power of the best land and the poorest land is far greater, where both receive the same treatment, than in any other portion of the state. The proportion of highly productive land is smaller also than in any other part of the state.

The region as a whole is a region of farm owners. A relatively small portion of the men whose principle means of livelihood is farming are entirely landless. There is a considerable percentage of rented land but the greater part of it, except a few tracts owned by mining companies, is rented by the owners of small farms who desire to extend their operations. Renting is chiefly a neighborly affair.

The Period of Readjustment.

The conditions in the Ozark border region are somewhat different, especially on the Perryville, Union, Owensville, Osage, Springfield and Bolivar soils. In the greater part of the areas of these soils the country is smoother, the soils less stony and as a whole more productive than the soils of the Plateau and Center areas. These areas also being adjacent to the Mississippi and Missouri river valleys on the east and north and to the smooth prairies on the west developed much more rapidly than did the more interior areas. Transportation to market was easier from the border region than from the interior both because of the shorter distance and because of smoother country. The people passed through the change from primitive stock raising on wild grass pasture to a later and different agriculture many years ago. There are very few areas within these soil belts where live stock have unrestricted range of the country or have had it for the past 15 to 25 years. On account of the smoother topography, better soils and better transportation the adjustment was easier here than in the interior parts of the region. Grain growing was profitable on both the upland and bottom soils so that when live stock could not be grown on the range the natural adjustment was to grain farming with curtailed live stock. Wheat became an important money crop—for many years the main money crop. This was supplemented by hog raising but relatively little attention was paid to cattle, horses or sheep.

Later the fruit industry began to develop in certain localities and in places, chiefly in the southwest corner and in Howell and Oregon counties, has become an important industry. Apples are grown to a considerable extent but strawberry growing has become more important, especially along the railway from Greene county southwestward. For several years most of the railway stations from Springfield

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to Carthage and from Monett to the Arkansas State line have been important shippers of berries. The industry is sufficiently important to cause the development of many local fruit-growing associations. Other small fruits, such as blackberries and raspberries are grown to some extent but they are not so important as strawberries. Peach growing has never been an important industry, chiefly on account of the climate. The trees do well. The crop of apples also is rendered very uncertain by late spring frosts. Strawberries are injured sometimes also.

Another phase of this fruit-growing industry is the growing of tomatoes for the local canneries. This industry is well developed in the Springfield region in the neighborhood of the railway. It has extended also into the Ozark Plateau region but has not become so well established there as it is in the southwestern border region.

In the northwestern, northern and eastern parts of the Ozark border the fruit growing industry has never been developed to any extent. It has remained a grain growing region since it abandoned its primitive stock raising system of farming.

A third readjustment to new conditions is beginning to take place in the southwestern Ozarks. This is the development of dairying. During the last five years dairying has attracted a great amount of attention in all the southwestern counties of the Ozark border. This has come as the natural development, following the intensive fruit farming, that has been going on for many years. It is very materially accelerated by the gradual waking up to the fact that no soil responds to manure better than the soils of the Ozark region and that the most effective machine for the manufacture of manure is the dairy cow when properly handled. The high price of dairy products for the last few years has been another important factor.

This interest in dairying has extended into the Ozark Plateau region, along railways. That part of the Plateau that lies near the railway has also had some development of the fruit industry but it omitted the grain growing stage on account of its soil. It is well adapted to dairying, however, and will develop it rapidly.

For a year or two there was an attempt to utilize the Springfield gravelly Black Oak soil in a few localities in Southwestern Missouri as a truck soil. A great deal of interest was aroused in potato growing. It was not maintained more than three years, however. The reason for the failure is not known. The potatoes were shipped to Texas. It was reported that they did not keep well in the Texas climate. Whether this was the real difficulty or not has never been determined. It was well demonstrated that potatoes would yield profitable crops on these soils. This occurred in the early nineties.

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AGRICULTURAL POSSIBILITIES OF THE REGION.

General Conditions.

The agricultural conditions of a region are the product of many factors such as soil, topography, climate, as well as the character of the population, the condition of communication and nearness to market. The fundamental factor is the soil. When soil, topography, and climate are favorable other conditions may be modified. When these are unfavorable the county cannot become a prosperous agricultural region.

The climatic conditions are practically uniform over the whole Ozark region. The natural factors, therefore, which cause variations of conditions, within the region, are soil and topography. These often vary together and where they do not do so the controlling factor, within certain broad limits, is soil. In general terms, therefore, it is true that the agricultural conditions of the Ozark region vary with the character of the soil. Some of the conditions determined by the character of the soil and other natural factors just named are favorable to agriculture while other conditions are unfavorable. Since this report concerns the soil chiefly the favorable and unfavorable conditions determined by the soil will constitute the greater part of what is said in the following pages, though some reference will be made to other factors.

Advantages of the Region.

Adaptability of the Soil.

While the soil, taking the region as a whole, is not a highly productive one, yet there is not another area in the state of anything like equal the size of the Ozark region that has a soil that is moderately well adapted to as many different phases of agricultural production. The fine part of the soil is very fine, making it readily adaptable to grasses and to a majority of the small cereals. It is true that tame grass pastures are not abundant in the region but that is due to the absence of any demand for them until recently and also to the low content of the humus supply. So far as the physical character of the soil is concerned it is adapted to most of the grasses, especially to the bunch grasses. All except the most extremely stony land will grow blue grass also where the humus supply is made adequate. The percentage of clay and silt in the soil is abundantly sufficient to constitute what the English people call a natural grass soil.

All of the stony and gravelly soils and the alluvial soils are natural clover soils. To these might be added the stony or gravel-free soils which have a brownish or reddish color and a reddish subsoil. Even on

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the flat silty uplands clover may be grown if a little care is taken in seeding it. On the other soils, except where clover and grain have been grown until the percentage of lime and humus has become low, the failure to get a stand of clover is the exception rather than the rule. There is every reason to think the gravelly and stony upland soils will grow alfalfa when the farmers have made up their minds to give the matter a thorough test.

The Ozark border region is second in importance in wheat raising only to the Missouri river bottom lands and the Loess soils adjacent to the river. The quality of the wheat is equal to any winter wheat grown in the Central West. The production per acre is not high but under proper rotation it could be materially increased.

The gravel and stones in the soil insure its perfect drainage while the fineness of the soil enables it to retain abundant moisture in seasons of average rainfall. Where the soil is well cultivated and kept supplied with humus it stands dry weather as well as the average soil of the other parts of the state. This insures its adaptability to corn growing when its fertility is mantained at a sufficient level. The yield of corn on the upland soils after they have been run in corn through the period of stump eradication is low. It will not average more than 20 or 25 bushels per acre. But where clover is grown and manure used on the land it will produce 35 bushels per acre, and, in good seasons, 40 to 45. Corn should not be grown on this land oftener than two years in five on account of its low fertility, not on account of its physical character. The bottom lands produce from 40 to 75 bushels per acre under average good conditions.

Its gravelly and stony nature insure its adaptability to a long list of vegetables. Vegetable gardens are, where they receive proper care and cultivation, almost always good and the range of products great. Potatoes, of both kinds, and beans do especially well.

The proportion of the total area of the country that, under a safe system of permanent agriculture, could be cropped in corn any one year is very small on account of the relatively small proportion of the area that is cultivated and the small proportion of that that should be cropped in corn in any year.

Water Supply.

The nature of the soil and of the underlying rocks causes the water supply of the region to be extraordinarily pure but over large areas it is not easily available. In the valleys of the large streams it is abundant. The perennial streams of the region are all large. There is no such thing as a brook in the whole region. The many springs that are found in the more hilly portions of the region supply a small

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stream that may run over the surface for a few thousand feet but it soon sinks beneath the gravel of its channel. On the uplands permanent "living" water must be sought in wells usually more than 100 feet deep. Good water can be reached in such wells practically everywhere in the region, but it is, in practically all cases, found in the rock, or on the contact between the rock and the loose clay and stones above it. A driven well is an impossibility in the whole region. Occasionally a small supply of water may be found in a shallow well dug at the head of a small hollow. Hundreds of the upland wells, however, that do not penetrate well into the solid rock remain dry or are dry in dry weather. Where wells are not dug or bored, water must be stored in reservoirs, either cisterns or open reservoirs. Such a supply is not satisfactory, though it is a common one. It is only on the creek bottoms or in the heads of hollows or sags in the upland that a good supply of water can usually be found above the solid bed rock.

Timber.

A considerable percentage of the area of the Ozark region will always be covered with timber. A relatively small part of it, however, will grow timber of very great commercial value. A great deal of the original growth of native timber has been used for commercial purposes but only for the cheaper grades of timber and the amount realized from it has been little more than the cost of cutting, preparing, and marketing it. It has not been an asset of very great value to the state. The pine region of the Ozark center counties makes the only exception to this general statement. It is of considerable value, however, to the farmers of the region. It furnishes an abundant supply of fuel and cheap building material; fence posts, framing timber, shingles, and ordinary oak lumber are cheap and abundant. The fencing and barn problems are easily solved and the fuel supply never fails. While these cannot be considered as important sources of income they are important in their reduction of the cost of farm equipment and of living.

Climate.

The climate of the region combined with the warm well drained soil is in many respects a decided advantage when compared with the surrounding country. Rainfall varies from about 38 inches on the Missouri river to 46 inches on the south line of the state, the maximum reaching about 50 inches in the southeastern part of the region where it abuts against the lowland region. The temperature varies from an annual average of 52 degrees along the Missouri to about 55 degrees in the southeastern corner of the area. These averages do not differ in any important respect from those in the same latitude east of this

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region. The character of the soil, however, is such as to make the winters dry and while the air is cold yet the absence of mud allows a freedom and ease of communication that is not found in other localities with a similar air temperature and rainfall. The advent of spring is earlier than in the neighboring regions not because of a more rapid increase of air temperature than in neighboring regions but chiefly because of the more ready response of the dry soil to the more direct rays of the sun. The pasture season begins earlier than in the clay soils of the east and persists until later in the autumn.

Structural Material.

The abundance of cheap timber for posts, framing, boxing, and firewood does not complete the list of cheap structural material found abundantly in the region. Limestone of excellent quality is found in many places and of fair quality for structural work about the farm practically everywhere. Limestone suitable for the manufacture of good building lime is especially abundant in the area of Springfield soils and in practically all other portions of the region a dark lime can be made. In modern farm construction cement is assuming a constantly increasing importance. The use of cement requires the use of gravel or broken stone. Gravel occurs in great abundance in practically every creek or river channel in the region and in many localities flint stones occur in great abundance already broken to sizes needed in concrete work. They retain an angularity as great as that of ordinary machine-broken stone. This is an excellent road material also and the supply is inexhaustible.

Disadvantages of the Region.

Topography.

Taking the region as a whole the topography constitutes a rather important hindrance to tillage. There is a relatively small area of very smooth land, though there are considerable areas which are not rough enough to hinder good cultivation. Like all the states lying south of the Illinoisan, Iowan, and Wisconsin, glacial boundaries Missouri has no very large areas of flat land. It is nearly all dissected more or less by streams. The Ozark region, being highest and having harder rocks, is cut the deepest and its roughness has not been reduced by the wearing down of the upland while the valleys were being cut out. Its angularity has not been softened by extensive weathering of its rocky bluffs. The hilly land of the Ozark region can be much more safely cultivated however than can land in North Missouri of equal roughness. The latter has a clay or silt soil without a sand or gravel con-

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stituent. It cannot absorb water rapidly. The rainfall, when heavy, runs off and takes with it a great deal of soil. The stony and gravelly soil of the Ozark region, on the other hand, absorbs a great deal of the rainfall. A very small portion of it runs off over the surface, even on rather steep slopes, except the relatively small areas where the soil is free from stone and gravel. While the more hilly part of the Ozark region is more deeply cut up by valleys than the more hilly portions of northern Missouri yet in the former the intricacy of the cutting is much less than in the latter. In North Missouri a forty acre tract may have and often does have as many as ten small ravines while in the Ozark region there is rarely more than three or four on an area of equal size.

Growth of Brush.

Practically the whole region was adapted to timber growth and considerable areas of it were actually covered with timber when settlement began. Where prairies existed they were not destitute of trees over large areas but were open grass lands with enough trees scattered over it to seed the land. The ground, even where trees did not exist, was full of roots. As soon as these got an opportunity to grow, growth commenced. By far the greater part of the area became covered with brush before it was broken by the plow. If it was not timber land it was at least brush land.

The universal method of preparing the land for the plow in the region is to cut the trees and brush, and leave the stumps and roots in place. The roots are never pulled out before the land is plowed. This is practically universal in the clearing up of the region. The trees and brush are chiefly oak—deep rooted trees and very tenacious of life. The roots remain alive in the soil for years and every year send up a vigorous crop of young sprouts. This continues for 5 to 8 years, or about as long as the ordinary stony upland soils produce fair crops of grain. It is not profitable to grow clover for hay on such land because of the impossibility of running a mower among the many stumps. Putting the land in pasture allows the brush to take possession of it in a surprisingly short time. The expense of keeping the brush down when the land is in pasture is heavy in proportion to the cost of the land and of the original clearing. It is protracted for many years, the stumps not dying so rapidly as when they are being bruised and broken during the cultivating season by the plow. Goats have been tried in a few places with apparent success. That, however, is expensive since first cost of the stock is considerable, also, and the possibility of selling the flock when their work is done rather doubtful. The average small farmer of the Ozark region has not yet seen his way clear

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to the adoption of this method of dealing with the matter. He cultivates the land in corn continuously for several years, cutting the sprouts once or twice each year until the stumps are dead. Occasional hickory or white oak roots remain alive and sprout after the land has been in cultivation for 20 years.

By the time the stumps are rotted out of the ground the land is pretty well exhausted of its humus. This is especially true if it be gravelly or stony hill land, though it applies to the bottom land also only to a slightly less degree. As soon as this condition is reached on the gravelly soils a crop of sassafras and persimmon sprouts are practically sure to spring up. Where continuous warfare is not waged against the sassafras especially, it will grow up in a very few years thick enough to exclude practically all other growth. These are difficult to eradicate as long as the land is in corn. A rotation with clover, however, and a few years with a June and August mowing will kill them out completely. As long as the land is kept well supplied with humus and has a crop of hay mowed from it every few years it will not be difficult to deal with both of these growths.

Baking of the Soil.

The fine grained constituents of the soils of the Ozark region are clay and silt. The ordinary term for both is clay. Taking the region over its soil is finer in grain than that of any other area of considerable size in the state. The almost universal system of grain farming in the region, especially while the land is new and rather productive, reduces the humus content rapidly. The lower this becomes, the greater is the tendency of the soil to puddle. When such a soil dries it becomes very hard. If it be worked while it is too wet it becomes very difficult to cultivate and the effect of one working at the wrong time will persist for many years. The farmer who is unfamiliar with this characteristic of the soil is often deceived and led to work his soil when too wet because of its normally loose loamy texture. This loamy texture is due to the gravel in it rather than sand, the finer constituent of the soil being finer than sand.

This characteristic is more pronounced in the upland soils than in the bottom land soils although the former are more stony or gravelly than the latter. The alluvial soils of the bottom land lost a considerable percentage of their finest grained constituents in the process of making. They are usually better supplied with humus also. While they were still occupied with their native growth the leaves were not burned off every autumn as they were on the hills. They decomposed into a layer of humus. The soil in the small hollows and narrow valleys that have no perennial streams as well as that in the larger valleys

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around the mouths of tributary hollows does not differ from the upland soils in this respect.

Fortunately the gravel constituent in these soils makes them much less intractable than they would be without them. Without the gravel to soften them up they would rebel absolutely against the treatment usually given them.

Cultivation of the Stony Land.

There is practically no type of soil in the Ozark region that is entirely free from loose stones. Some of the types are stone-free over most of their area and one or two are practically stone-free. The rule, however, is a more or less stony soil. Within the areas where stone is most abundant in the stony types, the soils occupying the two topographic extremes, the bottom lands and the flat topped ridges, are nearer free from stone than the other types. The bottom lands are apt to be stony along the outer margins, near the hill border, especially in the neighborhood of the mouths of hollows. These places, however, are usually gravelly rather than stony. The low bottom lands in the broader bottoms are stone free for several feet in depth of soil. The narrow dry hollows are often more stony than the hill lands. The flat topped ridges are never, or rarely, gravelly. They have usually stone-free silt or clay soils or else silt or clay soils with a varying proportion of "chunk rock," or flint stones, ranging up to a foot in diameter, very few of them being small. They, like the stones in all the soils, are angular, often with sharp edges. Beneath a silt layer of about a foot these upland flats are as stony as any of the region. It is only the soil layer that is stone free or partially so.

The farmer in the region must of necessity, cultivate stony or gravelly land. This stone when pure, fresh, and not disintegrated is as hard as any stone in existence. It wears tools, especially farming tools, rapidly. Horses wear their shoes rapidly and must be kept shod at all times. The larger stones in the soil make efficient plowing rather difficult. The ordinary breaking plow is apt to be thrown out of the ground very often by striking them. On some of the very stony land it is almost impossible to plow with any thing but a sharp pointed plow, that will slip off the stones when it strikes them without being thrown out of the ground.

The disadvantages arising from this condition can be overcome by picking the stones up and hauling them off the land. This is a rather expensive undertaking but not so much so as that of taking the stones from the stony glacial soils of the northern states. In the Ozark region a stone too large for a man to lift and place on a wagon practically never occurs on land that can be converted into farm land. In the

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northern states stones of such a size are very common. The clearing of the average stony land of the Ozark region of its stones can be done at a cost of from $5.00 to $20.00 per acre.

SYSTEMS OF FARMING ADAPTED TO THE REGIONS.

Systems of farming that are profitable in any particular region are determined by the nature of the country, its position or relation to market and to its climate.

The Ozark region is one of very pronounced characteristics. It is unlike any other area of equal, or approximately equal size in the world. Profitable farming in such a region must be definitely adjusted to it. It is not an area where loose and diverse methods with no relation to environment can be profitable. Several systems may be practiced in the region but each one must be definite in its adjustment.

In the following pages certain systems to which the region is adapted, as well as certain others to which it is not adapted, are discussed very briefly.

Grain Farming.

Grain farming on an extensive scale is an impossibility in any part of the Ozark region. The alluvial soils are all excellent grain soils but the proportion of these to the total area is too small to permit the production of a surplus of grain. The best of the upland soils produce fair crops of grain also, but the area of this is small. There can never be a surplus of corn, at least, produced in any part of the region after the adjustment of agricultural practice to the natural environment of soil and climate. There is such a large area that is adapted to grazing alone that the grain production can never run beyond the amount that the stock that is grown on the pastures will demand. The impression seems to prevail in many places that stock in the Ozark region does not need a great deal of winter feed. The facts are, however, that the winter feeding period is nearly as long as it is in any part of the state. When clover pastures are used it comes a little earlier in the spring and lasts until a little later in the fall than in the northern part of the state. The range grasses, however, do not appear any earlier than the tame grasses of the other parts of the state.

The nature of the soil is such that it cannot stand grain growing as continuously as the other soils of the state. On account of their loose texture and open porous character their humus supply is soon exhausted by continuous cultivation. Wheat growing has for many years assumed a high importance relative to the growth of the other grains. Corn and wheat are practically the only grains grown. As the agricultural practice adjusts itself so as to realize the greatest pos-

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sible profit wheat growing will probably decline because of an increased demand for corn to feed the stock that are being grown on the pastures.

Stock Feeding.

Stock feeding, especially cattle feeding and finishing, can never become an important industry on account of the small amount of corn land.

Hog feeding on a small scale and sheep feeding can be done with profit. The clover and alfalfa land will be effective aids in this branch of the industry.

Dairying.

There are several reasons why dairying is proving to be more profitable than any other branch of agriculture in the Ozarks. The first is:

Cleanliness.

Of all the area of the Central Mississippi Valley the Ozark area has greater freedom from mud. The universal presence of stones and gravel in the soil insures solid roads and good drainage under ordinary conditions. The spring thaws have nothing like the terrors and inconveniences that accompany them in the prairie region. The thorough drainage of the soil prevents the accumulation of surplus water during the winter and the consequent heaving of the soil. The latter is practically unknown except on the flat upland clay soils.

The absence of suspended matter from the water supply is another factor in the cleanliness of the region. The running water is universally clear except during the short flood periods of the streams and the banks and channels of the streams are lined with gravel. There are no unsightly mud-lined streams on which stock must depend for water. The artificial ponds on the uplands cannot be so favorably described but their existence is not necessary. Pure water may be obtained anywhere in the region in wells of no great depth.

Climate.

The mildness of the winters, including the great amount of sunshine, promotes the comfort and health of the stock and of the farmer as well. It lessens the cost of barns and to a certain extent of feed. The number of days during the winter when cows must be kept under shelter is relatively small. Shelters must be provided, however, but most of the farms, if they be rather large ones, grow part or all of their own building material.

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Roads.

The abundance of road material make it possible to build good roads all over the Ozark region at less than half the cost of the same roads elsewhere in the state. In fact over large areas the best of roads can be made by merely providing for drainage. It is usually unnecessary to provide drainage to prevent mud but it is necessary to prevent washing on the hill sides. The roads are good throughout the year, or can be made so. There is no period during the year when the farmer will be unable to get over the roads with his dairy products.

Legumes.

The ease with which leguminous crops are grown, the adaptability of the soil to grass and the relatively small amount of grain grown make dairying better adapted to the cropping system than any other system of farming except that of stock raising. Dairying will yield a much greater profit than merely raising cattle for the feeder market.

Manure.

The astonishing response that the Ozark soils make to barn yard manure is one of the most important facts to be considered in the farming system. No other system of farming produces as great a quantity of manure per acre of land as does dairying. The physical character of the average Ozark soil, when the stones are removed from it, is well nigh ideal. It is well drained and easily warmed up. Its content of clay insures the retention of moisture and of fertilizer. The soil as a rule does not leach. Soil conditions make the use of manure extremely profitable.

Transportation.

The facilities for transportation over the whole central part of the region are poor. This makes dairying impossible since local markets do not exist. Dairying in the region would have to depend on the great markets. On account of the lack of transportation facilities these cannot be reached. In the Ozark border region, however, and along the three railway lines that cross or approach the central part this system of farming is bound to develop rapidly. For the rest of the region a return to the stock raising of several years ago will prove to be most profitable.

Fruit Growing.

A great deal of the soil of the Ozark region is adapted to the growth of fruit trees, vines and plants. Those best adapted to such plants are the stony and gravelly soils in which the fine grained con-

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stituent does not run extremely low. What the lower limit is has not been determined. The limit of profitable growth seems to be where there is not enough clay to prevent leaching. When this occurs the soil does not produce any crop profitably and fruit trees do not constitute an exception. All those gravelly soils having a brown color and a red subsoil seem to be well adapted to the growth of fruit trees and plants. Other soils having blue clay subsoils will often grow the trees fairly well but will not produce good fruit. The clay or silt soils underlain by a layer of cemented gravel or hardpan does not produce either tree or fruit profitably except the small berries and that grown on short lived trees. The growth of berries does not seem to be conditioned by the soil to so great an extent as that of the tree fruits. The tree fruits must have a soil capable of maintaining a tree for many years while the berries may be grown on any soil that has a moderate fertility. The only condition affecting the distribution of the growth of berries in the region is the facility for cheap transportation to market.

The growth of fruit is, however, not dependent solely on the possibility of growing fruit trees. The latter will flourish where the soil conditions are favorable, even though the climatic conditions may not be favorable for the production of fruit. The extremes of temperature in the Ozark region and the frequent occurrence of spring frosts make the growth of peaches and apples a somewhat uncertain industry. In years when the fruit is not killed by late frosts the production of fruit is heavy and the quality good. The frequence with which late frosts occur make the growth of a crop of tree fruits in practically the whole region too uncertain to warrant making it an exclusive industry. It is profitable and probably always will be profitable to carry it on as one part of a varied system of farming.

The southeastern quarter of the region is less subject to late frosts than the rest. Too much emphasis cannot well be placed on the fact that the limitation of the growth of fruit in the Ozark region is not determined, for the region as a whole, by the failure of the soil to grow vigorous trees or to produce good, well colored fruits, but by the frequent occurrence of late frosts in the spring. In addition to this, peaches are often winter killed, owing to the frequent periods of open, warm sunny weather during winter, which cause the buds to swell. They are then killed by the almost invariable severe cold snap in February. The southeastern quarter of the region is more nearly exempt from this condition than the other parts of the region.

The Ozark region is one of stronger climatic contrasts than the northern part of the state. Warm weather during winter warms up the soil rapidly owing to its thorough drainage and the abundance of loose

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stone in the soil while cold weather has the opposite effect due to exactly the same soil character. The warm weather of winter is warmer normally and the cold weather is colder than in the clay soils of North Missouri because of the character of the soil. In other words, it is, so far as temperature of the air and soil is concerned, a region with a tendency toward arid conditions; so far as moisture is concerned, however, with its heavy rainfall and its clay subsoil it differs widely from such a region.

Stock Raising.

The original industry of the region was stock raising. Cattle were sold off the range as grass fed cattle. Corn was fed to them only during the winter and then only enough to get them through the winter. The destruction of the range has caused the abandonment, to a great extent, of this industry and no other system of farming has been found that would apply, as well as the old one, to the Ozark Center and the Ozark Plateau areas. Fruit growing has been tried but owing to the climate it has not been found profitable except in small local areas. Cattle feeding cannot be developed because of the lack of an abundant supply of grain. In a large part of the region dairying cannot become an important industry for some time to come because of the lack of facilities for transportation. Because of the small amount of grain soil and the large area of pasture land the two systems of farming that can be carried on are stock raising, not including hogs, except in small numbers, and dairying. This being the case the only feasible system at present for a large part of the area is stock raising. There must be a return to grazing. In order to do this the land must be reset to grass. The brush and timber will have to be killed out and the land brought back to its original condition. This will be found to be the more profitable system in the area of the Plateau, especially. On account, however, of the adaptability of the Ozark center area to the growth of pine timber, the growth of that and the industries based on it will be found to be more profitable than any system of farming except the small amount of grain farming that is and will continue to be carried on in its valleys.

On account of the greater proportion of tillable soil, the smoother topography, better transportation facilities and denser population the farming system on the Ozark border soils is rapidly adjusting itself to dairying and incidental fruit growing, especially in the main area of Springfield soils. Elsewhere in the Ozark border general farming is the rule and is carried on with reasonable profit.

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To sum up then: The Ozark Center will gradually evolve into a forested region with grain farming in the valleys and limestone basins. The farming population will be very sparse and its products will be locally consumed by an industrial population depending upon the timber for its livelihood. The stock raised will be sheep, hogs, cattle, horses and mules.

The Ozark Plateau will be found to be better adapted to grazing than to timber growth. It does not grow pine and its oak timber is of very slow growth. Fruit growing will be carried on incidentally along the railways along with dairying. The more important industry, however, will be stock raising.

The Ozark border will engage in general farming, dairying and fruit raising. Dairying will gradually assume greater importance than either of the others and probably of both of them.

PLATE NO. 1.

A series of geological cross sections across the state from A at the southwest corner of McDonald county to C on the Mississippi river at Ste. Genevieve.

The first section, comprising the top series; A to B, B to C, C to D, shows the region as it is supposed to have been after the rocks had been deposited, but before they had been bowed upward or eroded.

The second section, comprising the middle series of three sections, shows the region after it was bowed upward, but before it was eroded. A heavy line down through this section shows approximately the position of the existing surface in the region and the relation of the amount that has been worn off to that which is left above sea level.

The third section, comprising the last series of three sections, shows the profile of the existing surface, its relation to the geological formations and the relation of the various soil groups to both.

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RESEARCH BULLETIN NO. 4

UNIVERSITY OF MISSOURI

COLLEGE OF AGRICULTURE

AGRICULTURAL EXPERIMENT STATION

Digestion Trial With Two Jersey Cows on Full Ration and on Maintenance

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COLUMBIA, MISSOURI
October, 1911

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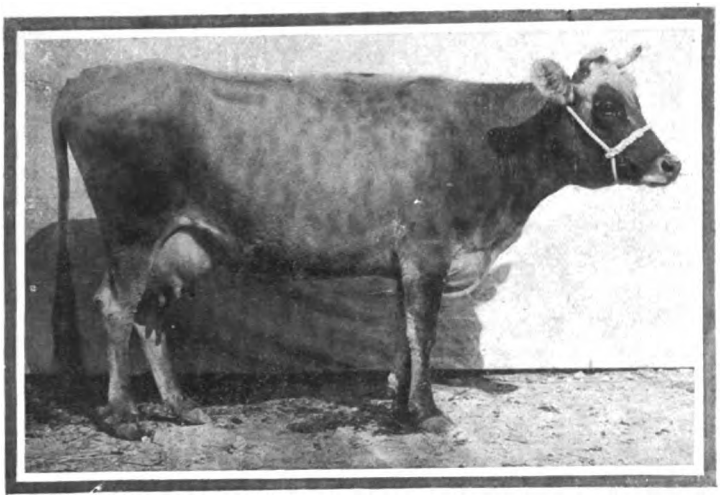
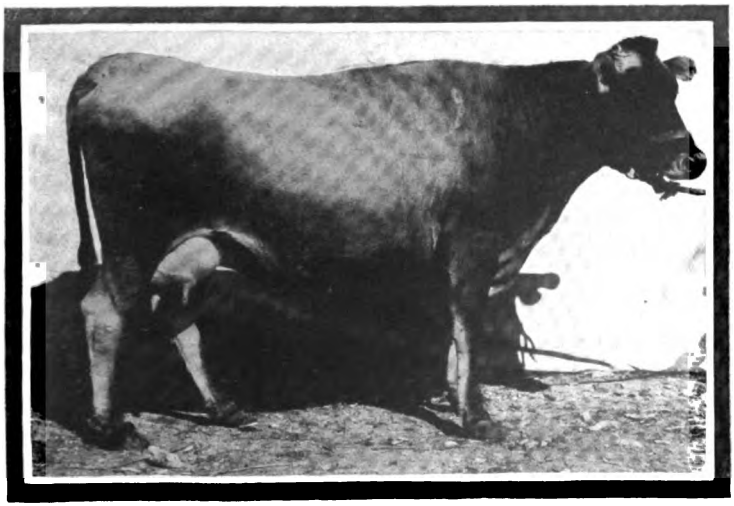


NO. 27, PEDRO'S RAMAPOSA 181160



NO. 62, PEDRO'S ELF 197242

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A DIGESTION TRIAL WITH TWO JERSEY COWS ON FULL RATION AND ON MAINTENANCE.

C. H. ECKLES.

In the course of an investigation already reported[1] digestion trials were conducted with two Jersey cows covering a period of 10 days when on full rations and again when on maintenance. These cows were registered animals known as Pedro's Ramapose 181160 and Pedro's Elf 197242. Table I gives data regarding the history of these animals.

TABLE 1.

	No. 27	No. 62
Date of birth	Sept. 4, 1902	May 11, 1903
Age at first calving	29 mo.	18 mo.
Lbs. Milk first lactation period	4552	878
Lbs. fat first lactation period	238.8	44.1
No. days in milk	337	131
Lbs. milk second lactation period	7174	3189
Lbs. fat second lactation period	377	114.8
No. days in milk	365	232
Lbs. milk third lactation period	8522	3188
Lbs. fat third lactation period	469.9	169.3
No. days in milk	365	321

The first digestion trial covered 10 days beginning December 27, 1907. The chemical work was done under the direction of Dr. F. F. Trowbridge, of the Department of Agricultural Chemistry.

The cows were about three months in milk at the time and practically at their maximum production. The digestion trial was carried out in the usual manner. The grain and hay ration for each day for each animal for the entire period was weighed out at the

[1]Research Bulletin No. 2, Mo. Exp. Station.

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beginning and a sample taken of each constitutent of the ration. It is not possible to do this with the silage since it will not keep outside the silo. For this reason the silage was weighed out from the silo at each feeding and a composite sample for chemical analysis made by taking a small portion at random from each feed and placing it in a tight glass jar in which sufficient chloroform had been placed to prevent spoiling. The cows were kept in the barn and watered in the manner to be followed during the digestion trial for a week preceding, in order that they might be accustomed to the routine.

Three attendants were provided for each cow, working in 8 hour shifts to collect the dung and urine. A common grain shovel was used for the former and a tin vessel about the size of an ordinary milk pail with a wooden handle was used for collecting the urine. None of the excreta was lost during the 10 days covered by the digestion trial.

Table 2 gives the ration received daily by the two animals.

TABLE 2.

RATION FED PER DAY.

Digestion Trial on Full Ration.

	No. 27		No. 62	
	Lbs.	Kilos	Lbs.	Kilos
Alfalfa Hay	9.0	4.082	6.0	2.585
Silage	35.0	15.875	22.0	10.115
Corn	6.28	2.857	4.0	1.814
Bran	3.14	1.406	2.0	.907
Oilmeal	1.57	.726	1.0	.454

This was the same ration as used throughout the entire investigation of which the digestion trial was one part. It consisted of alfalfa hay of the best quality, corn silage made from well matured corn, and a grain mixture of corn meal 4 parts, wheat bran 2 parts and oilmeal 1 part. The ration of both animals was made up in the same proportion with the only difference being the quantity given which had been adjusted during the several weeks preceding to maintain the cows at uniform weight. It will be noted that No. 27 re-

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ceived considerably more than No. 62 which is accounted for by her larger production of milk.

Table 3 gives the chemical analysis and tables 4 and 5 give the amounts of the several constituents in the ration received by the two cows.

TABLE 3.

PERCENTAGE COMPOSITION OF DAILY RATION.

Digestion Trial December 27, 1907 to January 5, 1908.

NO. 27 AND NO. 62.

	Dry Matter	Ash	Nitrogen	Protein	Crude Fibre	Nitrogen free Extract	Fat
Alfalfa Hay.	94.16	8.225	2.030	12.68	36.350	35.33	1.560
Silage.....	29.29	1.812	.319	1.99	7.082	17.58	.825
Corn.....	85.04	1.193	1.275	7.97	1.897	69.99	3.993
Bran.....	89.51	6.668	2.328	14.55	8.487	55.05	4.749
Oilmeal....	90.89	5.343	5.498	34.36	7.975	36.47	6.736
Refused....	94.53	7.013	1.461	9.13	41.673	34.77	1.943

TABLE 4.

COMPOSITION OF DAILY RATION FED NO. 27.

Weights in grams.

	Am't Fed	Dry Matter	Ash	Nitrogen	Protein	Crude Fibre	Nitrogen free Ext.	Fat
Alfalfa Hay.	4082.0	3843.6	335.74	82.86	517.88	1483.81	1442.17	63.68
Corn Silage..	15875.0	4649.7	287.66	50.64	316.50	1124.27	2789.83	130.97
Corn.....	2857.0	2429.5	34.08	36.43	227.69	54.20	1999.61	114.08
Bran.....	1406.0	1258.5	93.75	32.73	204.56	119.33	774.00	66.67
Oilmeal....	726.0	659.8	38.79	39.93	249.50	57.90	264.77	48.90
Total.....	24946.0	12841.1	790.02	242.59	1516.13	2839.51	7270.38	424.30
Refused....	73.2	69.1	5.13	1.06	6.68	30.50	25.45	1.42
Total Rat'n.	24872.8	12772.0	784.89	241.53	1509.45	2809.01	7224.93	422.88

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TABLE 5.

COMPOSITION OF DAILY RATION FED NO. 62.

Weights in grams.

	Amt. Fed	Dry Matter	Ash	Nitrogen	Protein	Crude Fibre	Nitrogen free Extract	Fat
Alfalfa Hay.....	2585.0	2434.0	212.62	52.48	328.00	939.65	913.28	40.33
Corn Silage...	10115.0	2962.6	183.28	32.27	201.69	716.34	1778.21	83.45
Corn.....	1814.0	1542.6	21.64	23.13	144.56	34.41	1269.62	72.43
Bran.....	907.0	811.8	60.48	21.11	131.94	76.98	499.30	43.07
Oilmeal....	454.0	412.6	24.26	24.96	156.00	36.21	165.57	30.58
Total.....	15875.0	8163.6	502.28	153.95	962.19	1803.59	4625.98	269.86
Refused...	60.7	57.4	4.26	.89	5.54	25.30	21.11	1.18
Total Ration..	15814.3	8106.2	498.02	153.06	956.65	1778.29	4604.87	268.68

Tables 6 and 7 record the amount of milk by milkings for each animal and the totals.

TABLE 6.

YIELD OF MILK AND FAT.

NO. 27.

Date	A. M.			P. M.		
	Lbs. Milk	Per cent Fat	Lbs. Fat	Lbs. Milk	Per cent Fat	Lbs. Fat
Dec. 26.....	.....	.....	.....	12.2	6.0	.7320
27.....	13.7	5.8	.7946	12.4	5.4	.6696
28.....	15.0	5.1	.7650	12.1	4.4	.5324
29.....	16.1	5.0	.8050	11.9	5.6	.6664
30.....	15.2	4.8	.7296	11.3	5.3	.5989
31.....	15.7	4.3	.6751	11.2	5.7	.6384
Jan. 1.....	15.0	5.4	.8100	12.1	5.1	.6171
2.....	14.3	5.1	.7293	12.3	5.3	.6519
3.....	14.3	5.2	.7436	11.7	5.5	.6435
4.....	15.0	5.7	.8550	11.1	4.6	.5106
5.....	15.5	5.2	.8060	.....	.....	.....
	149.8	5.14	7.7132	118.3	5.29	6.2608

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TABLE 7.

YIELD OF MILK AND FAT.

NO. 62.

Date	A. M.			P. M.		
	Lbs. Milk	Per cent Fat	Lbs. Fat	Lbs. Milk	Per cent Fat	Lbs. Fat
Dec. 26	.....	.....	.....	6.1	5.4	.3294
27	7.0	5.6	.3920	5.9	5.0	.2950
28	5.2	3.1	.1612	8.1	5.8	.4988
29	7.7	6.0	.4620	5.6	5.2	.2912
30	7.4	4.9	.3626	6.8	4.7	.3196
31	7.0	5.6	.3920	6.4	5.6	.3584
Jan. 1	7.1	5.1	.3621	5.9	5.0	.2950
2	7.2	5.0	.3600	6.2	4.8	.2976
3	7.4	5.5	.4070	6.1	4.9	.2989
4	6.9	4.6	.3174	5.8	5.5	.3190
5	7.2	5.6	.4032	.....	.....	.....
	70.1	5.16	3.62	63.4	5.2	3.30

Table 8 gives the chemical composition of the milk of each cow and the total of each constituent in both pounds and kilos.

TABLE 8.

COMPOSITION OF MILK.

NOS. 27 AND 62.

	Percentage Composition		Constituents in Lbs. and Kilos			
	No. 27	No. 62	No. 27		No. 62	
			Lbs.	Kilos	Lbs.	Kilos
Total Milk	.....	.....	268.1	120.61	133.5	60.55
Total Nitrogen	.63	.64	1.69	.76	.85	.39
Total Protein	4.02	4.08	10.78	4.85	5.45	2.47
Fat	5.10	5.10	13.67	6.15	6.81	3.09
Sugar	5.20	5.20	13.94	6.27	6.94	3.15
Ash	.77	.81	2.06	.93	1.08	.49

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Table 9 records the daily weights of dung and of urine for each animal and in table 10 is found the result of the analyses.

TABLE 9.

WEIGHTS OF DUNG AND URINE DIGGESTION TRIAL ON FULL RATION.

Date	No. 27		No. 62	
	Weight of fresh dung Kilos	Weight of fresh urine Kilos	Weight of fresh dung Kilos	Weight of fresh urine Kilos
Dec. 27, 1907.....	30.245	8.092	12.854	4.632
Dec. 28, 1907.....	28.011	9.702	15.559	4.390
Dec. 29, 1907.....	30.110	7.159	14.591	4.642
Dec. 30, 1907.....	35.608	8.511	14.599	5.440
Dec. 31, 1907.....	32.728	6.666	14.153	4.626
Jan. 1, 1908.....	29.935	8.993	15.044	4.451
Jan. 2, 1908.....	31.572	7.589	14.313	4.990
Jan. 3, 1908.....	28.801	6.643	13.973	4.404
Jan. 4, 1908.....	26.638	8.607	14.637	5.376
Jan. 5, 1908.....	28.237	8.482	14.963	4.648
Average.....	30.182	7.999	14.469	4.760

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TABLE 10.

ANALYSES OF DUNG AND URINE DIGESTION TRIAL ON FULL RATION.

	Composite Sample		Average of Daily Samples	
	No. 27	No. 62	No. 27	No. 62
Dung				
Dry Matter.....	14.95	19.44	.....	.....
Moisture.....	85.05	80.56	.....	.....
Nitrogen.....	0.33	0.417	0.322	0.425
Protein.....	2.062	2.606	2.013	2.576
Fat.....	0.463	.0746	.....	.....
Crude Fibre.....	4.298	5.666	.....	.....
Ash.....	1.574	2.029	.....	.....
N. free Ex.....	6.552	8.393	.....	.....
Urine				
Dry Matter.....	7.16	8.03	.....	.....
Moisture.....	92.84	91.97	.....	.....
Nitrogen.....	0.799	1.024	0.783	1.036

The nitrogen was determined separately in both dung and urine daily. A composite sample of each was made by placing an amount equal to 1 part in 50 of the total in an air tight jar with sufficient chloroform added to prevent fermentation. The jars were kept in a temperature of about 5° C. The nitrogen determinations of both urine and dung in the composite sample agrees closely with the averages of the daily analyses.

Tables 11 and 12 give the total amounts of the different nutrients consumed, the amount excreted in the dung and the per cent digested. It will be noted here that while there is some difference regarding the digestibility of the different constituents the average figure was almost exactly the same for both animals.

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TABLE 11.

SUMMARY RESULTS AVERAGED BY DAYS.

NO. 27.

Weights in grams.

	Consumed	Excreted in Dung	Per cent Digested
Protein.....	1509.44	622.51	58.75
Fat.....	422.88	139.74	66.95
Crude Fibre.....	2809.01	1297.22	53.82
Nitrogen-free Extract.	7224.93	1977.53	72.62
Total.....	11966.26	4037.00	66.27

TABLE 12.

SUMMARY OF RESULTS AVERAGED BY DAYS.

NO. 62.

Weights in grams.

	Consumed	Excreted in Dung	Per cent Digested
Protein.....	956.65	377.13	60.58
Fat.....	268.68	107.94	59.82
Crude Fibre.....	1778.30	819.81	53.89
Nitrogen-free Extract.	4604.87	1214.38	73.62
Total.....	7608.50	2519.26	66.95

Table 13 gives a comparison of the per cent of the several constituents actually digested and the amount that would be digested according to the average digestion coefficients as given by Jordan.[1]

[1]The Feeding of Animals, p. 427.

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TABLE 13

COMPARISON OF AVERAGE AND ACTUAL DIGESTION COEFFICIENTS.

NO. 27 AND NO. 62.

On Full Ration.

	Protein		Crude Fibre		Nitrogen free Extract		Fat	
	Average	Actual	Average	Actual	Average	Actual	Average	Actual
No. 27.....	70.2	58.75	53.9	53.82	76.6	72.62	78.0	66.95
No. 62.....	70.2	60.58	53.9	53.89	76.6	73.62	78.0	59.82

Using average figures No. 27 should have digested 70.81 per cent of the ration while she digested 66.27 as found by trial.

The figures for No. 62 by average coefficients is 70.79 while by trial the figure was 66.95.

On Maintenance.

	Protein		Crude Fibre		Nitrogen free Extract		Fat	
	Average	Actual	Average	Actual	Average	Actual	Average	Actual
No. 27.....	69.4	67.32	52.7	55.33	74.6	82.12	77.0	73.17
No. 62.....	68.7	65.54	53.8	52.06	75.4	80.99	76.7	73.92

When on maintenance No. 27 digested 73.79 per cent and No. 62 72.19 per cent of the entire ration. The average digestion coefficient for the same ration are 69.1 for No. 27 and 69.7 for No. 62.

The first half of the table gives this comparison for the digestion trial made while the cows were on full ration and the second half for the digestion trial made for the same animals when on maintenance. The columns headed "Average" is the digestion coefficient of this ration calculated by applying average figures of digestibility to the data given in Tables 4 and 5. The columns headed "Actual" gives the coefficient of digestion as actually determined for No. 27 and No.62. When the cows were on full ration it will be noted the coefficient was decidedly lower for both cows than the average with the exception of the crude fibre. The data for the second digestion trial indicates

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the same cows on a maintenance ration of the same composition as in the first were able to show a coefficient of digestibility fully as high as the averages ordinarily used. This seems to indicate the depression in the digestion coefficient with both animals when on the full ration was due to the amount of the ration consumed. When both were on maintenance No. 27 had on the whole a higher digestion coefficient than No. 62 while on a full ration the reverse was true. That is, on a full ration when No. 27 was consuming 50% more feed than No. 62 her coefficient of digestion was lower, while when both were dry and farrow she was able to digest a slightly higher per cent of her food. The general tendency of the digestion trials carried on at this Experiment Station with steers by Dr. P. F. Trowbridge the results of which have not as yet been published also indicate that the plane of nutrition has a decided influence on the digestion coefficient.

Since most of the digestion trials upon which the average digestion coefficients are based were made with animals at or near maintenance conditions it is not surprising that animals on full ration are not able to make as good use of the food as those on a lower plane of nutrition. While the results given are entirely too few upon which to base a conclusion it at least is justifiable to raise the question if the digestion coefficients for use in making calculations regarding the feeding of dairy cows should not be determined by using cows that are producing large quantities of milk and for this reason are receiving a heavy ration rather than by making use of coefficients of digestibility from trials with steers under maintenance conditions.

Table 14 gives the nitrogen balance. The outgo of nitrogen in milk, urine and dung agrees closely with that taken in by the food in both animals.

TABLE 14.

DAILY NITROGEN BALANCE.

Weights in grams.

	No. 27	No. 62
Dung	99.60	60.34
Urine	63.90	48.74
Milk	76.00	39.00
Total	239.50	148.08
Consumed in Feed	241.53	153.06

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DIGESTION TRIAL ON MAINTENANCE

Abuut 13 months later, a digestion trial was again made with the same two cows. This time they were dry and farrow. The trial lasted 10 days beginning January 28, 1909. No. 62 had been on maintenance 150 days and No. 27, 90 days at the beginning of this trial. Data regarding the maintenance period is given in detail in another part of this publication. The ration fed the animals was the same as given when in milk at the time of the first trial. The same grain mixture was used and practically the same ratio between the grain, hay and silage was maintained. For each pound of grain, each cow consumed, she also received one pound of alfalfa hay and four pounds of silage. The amount given was of course such as was found by trial during the preceding months to be necessary for maintenance.

Table 15 gives the ration fed and Table 16 the chemical composition of each constituent of the ration.

TABLE 15.

RATION FED PER DAY, DIGESTION TRIAL ON MAINTENANCE.

	No. 27		No. 62	
	Lbs.	Kilos	Lbs.	Kilos
Alfalfa Hay	3.2	1.451	2.8	1.270
Silage	12.8	5.806	11.2	5.080
Corn	1.83	.830	1.6	.726
Bran	.91	.415	.8	.363
Oilmeal	.46	.207	.4	.181

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TABLE 16.

PERCENTAGE COMPOSITION OF RATION FED, DIGESTION TRIAL ON MAINTENANCE.

NO. 27 AND NO. 62.

	Dry Matter	Ash	Nitrogen	Protein	Crude Fibre	N. free Extract	Fat
Alfalfa Hay.	94.21	8.960	2.250	14.06	32.97	35.84	2.380
Silage.....	30.192	1.869	.227	1.42	7.00	18.23	1.676
Corn.....	94.07	1.190	1.415	8.84	2.03	78.24	3.770
Bran.....	90.285	7.293	2.335	14.59	10.25	53.50	4.645
Oilmeal....	93.45	5.540	5.440	34.00	8.03	38.57	7.310

Tables 17 and 18 give the total amounts of each constituent of the ration received by the two cows.

TABLE 17.

COMPOSITION OF RATION, TEN DAYS DIGESTION TRIAL ON MAINTENANCE.

NO. 27.

Weights in grams.

	Dry Matter	Ash	Nitrogen	Protein	Crude Fibre	N. free Extract	Fat
Alfalfa. ...	13674.49	1300.53	326.58	2040.79	4785.56	5202.14	345.45
Silage	17529.49	1085.13	131.80	824.44	4064.17	10584.25	973.08
Corn	7807.20	98.72	117.39	733.38	168.41	6493.92	312.77
Bran	3743.04	302.35	96.80	604.87	424.94	2218.00	192.57
Oilmeal ...	1937.13	114.84	112.77	704.79	166.45	799.52	151.53
Total	44691.55	2901.57	785.34	4908.27	9609.53	25297.86	1975.40

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TABLE 18.

COMPOSITION OF RATION, TEN DAYS DIGESTION TRIAL ON MAINTENANCE.

NO. 62.

Weights in grams.

	Dry Matter	Ash	Nitrogen	Protein	Crude Fibre	N. free Extract	Fat
Alfalfa.....	11965.14	1137.96	285.76	1786.00	4187.35	4551.86	302.27
Silage.....	15338.14	949.49	115.32	720.75	3556.14	9261.20	851.44
Corn.....	6827.04	86.36	102.69	641.81	147.33	5678.19	273.60
Bran.....	3276.17	264.64	84.73	529.56	371.94	1941.35	168.55
Oilmeal.....	1695.56	100.52	98.70	616.88	145.69	699.81	132.63
Total.....	39102.05	2538.97	687.20	4295.00	8408.45	22132.41	1728.49

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Table 19 gives the weights of dung and urine by days.

TABLE 19.

Weight in pounds.

Date	No. 27		No. 62	
	Weight of fresh dung Kilos	Weight of fresh urine Kilos	Weight of fresh dung Kilos	Weight of fresh urine Kilos
Jan. 29, 1909	6.996	3.683	6.732	3.842
Jan. 30, 1909..	7.471	3.279	5.765	3.847
Jan. 31, 1909..	5.419	2 950	4.851	4.079
Feb. 1, 1909.....	5.905	3.205	4.472	3.742
Feb. 2, 1909.....	6.025	3.609	4.789	3.021
Feb. 3, 1909.....	7.017	3.377	5.073	3.160
Feb. 4, 1909.....	6.528	3.667	5.200	3.163
Feb. 5, 1909.....	6.364	3.277	6.649	3.942
Feb. 6, 1909.....	6.777	3.743	6.010	3.586
Feb. 7, 1909.....	7.765	4.348	6.149	4.305
Average.....	6.627	3.514	5.569	3.669

The excreta was handled in the same manner as described for the first trial. Only composite samples were taken in this case since the analyses of the daily and of the composite samples had been found in the first trial to check closely. The analyses of the composite samples of dung and urine is found in Table 20.

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TABLE 20.

COMPOSITION OF DUNG AND URINE DIGESTION TRIAL ON MAINTENANCE.

	Composite Sample	
	No. 27	No. 62
Dung		
Dry Matter	18.998	20.837
Moisture	81.002	79.163
Nitrogen	0.387	0.424
Protein	2.419	2.650
Fat	0.799	.807
Crude Fibre	6.473	7.217
Ash	2.487	2.630
Nitrogen free Extract	6.82	7.531
Urine		
Nitrogen	1.523	1.360

The nitrogen balance as given below shows a reasonably close agreement.

	Weights in Grams No. 27	Weights in Grams No. 62
Dung	25.65	23.61
Urine	53.52	49.90
Total	79.17	73.51
Consumed in Feed	78.53	68.7

Tables 21 and *22* give a summary of the results of this digestion *trial.*

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TABLE 21.

SUMMARY OF DIGESTION TRIAL ON MAINTENANCE.

NO. 27.

	Consumed Grams	Excreted in Dung		Per cent Digested
		Per cent	Weight Grams	
Protein	4908.27	2.419	1604.19	67.32
Fat	1975.40	.799	529.92	73.17
Crude Fibre	9609.53	6.473	4293.08	55.33
N. free Ex.	25297.86	6.82	4523.23	82.12
Total	41791.06	.....	10950.42	73.79

TABLE 22.

SUMMARY OF DIGESTION TRIAL ON MAINTENANCE.

NO. 62.

	Consumed Grams	Excreted in Dung		Per cent Digested
		Per cent	Weight Grams	
Protein	4295.0	2.65	1480.25	65.54
Fat	1728.49	.807	450.78	73.92
Crude Fibre	8408.45	7.217	4031.34	52.06
N. free Ex.	22132.41	7.531	4206.18	80.99
Total	36564.35	.....	10168.55	72.19

The second column, headed per cent, in both tables refers to the composition of the dung, the weights of which are found in Table 19. The third column is the weight of the constituents excreted and the fourth column the per cent digested. No. 27 shows a higher

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per cent of each constituent digested with an average of 7.5 more for the entire ration when on maintenance. No. 62 also has digested a larger per cent of each constituent and an average of 5.24 per cent higher when on maintenance.

Table 23 is included as a matter of interest. It gives the amount of water consumed daily by each cow while on full ration producing and again when dry and on maintenance. It illustrates the increased demands for water by the cow in milk and consuming a large ration.

TABLE 23.

WATER CONSUMED DURING DIGESTION TRIALS.

Weights in Pounds.

Day	No. 27		No. 62	
	First	Second	First	Second
1	94	0	68	23
2	82	0	30	0
3	77	20	37	0
4	81	23	43	28
5	82	17	30	17
6	82	15	46	0
7	73	20	32	30
8	77	18	46	18
9	65	17	40	0
10	60	17	29	12
Average	77.3	14.7	40.1	12.8

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SUMMARY AND CONCLUSIONS.

Complete data is given of a digestion trial made with two Jersey cows when at the maximum yield of milk and repeated with the same animals when dry and on maintenance. The ration used was the same in both trials except in quantity.

When on full ration the per cent digested was lower with both animals and for each constituent of the ration than the average figures in common use.

The cow which received the most liberal ration digested 66.27 per cent of the entire ration. According to the digestion coefficients in common use she should have digested 70.81 per cent. The same cow on maintenance digested 73.79 per cent of the ration. The average figures for the same ration are 69.1 per cent. The second cow receiving about 50 per cent less feed, during the trial when in milk digested 66.95 per cent of the ration. On maintenance the same animal digested 72.19. According to the average figures in use she should have digested 70.79 per cent in the first trial and 69.7 for the second.

These results suggest that the average digestion coefficients in use are somewhat high as applied to cows producing large quantities of milk which requires a heavy ration and that accurate figures for this purpose should be obtained from experiments with cows in milk.

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RESEARCH BULLETIN NO. 5.

UNIVERSITY OF MISSOURI

COLLEGE OF AGRICULTURE

AGRICULTURAL EXPERIMENT STATION

Maintenance Trials With Five Jersey Cows

COLUMBIA, MISSOURI
October, 1911

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MAINTENANCE TRIALS WITH FIVE JERSEY COWS.

C. H. ECKLES.

The two cows, data concerning which is given in connection with a report on digestion trials,[1] and three other cows of the same breed were kept for periods of from 120 to 180 days on maintenance. The special object in determining the maintenance of two of the animals, Nos. 27 and 62, has been given in another publication.[2] The other three animals were used in another investigation having for its object the determination of the comparative cost of producing solids in the form of milk and live weight on the animal body by the same animals. Details of this latter investigation have not as yet been put in print.

The five Jersey cows used were registered animals of that breed and were all half-sisters, having the same sire. Details regarding two of these animals will be found in another publication.[1] Below is given data regarding the other three. The figures given for the yield of milk and the yield of fat is for the period of lactation, 365 days in length, which closed shortly before the maintenance trial was begun.

	No. 4	No. 63	No. 43
Name and Number	Pedro's Alphea Elf 168587	Pedro's Grace Briggs 197836	Miss Missouri 181159
Age	8 yrs. 6 mo.	5 yrs. 6 mo.	6 yrs. 3 mo.
Weight	792 lbs.	889 lbs.	736 lbs.
Yield Milk	6773 lbs.	6077 lbs.	8137 lbs.
Yield Fat	376 lbs.	370 lbs.	398 lbs.

The ration fed four of the animals, Nos. 27, 62, 4, and 63, was exactly the same except the quantity was varied as was found necessary to maintain a uniform weight. The ration used was of the same composition as given while the same animals were producing

1. Research Bul. No. 4 Mo. Exp. Sta.
2. Research Bul. No. 2 Mo. Exp. Sta.

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milk since it was desired to determine maintenance requirements in terms of the ration fed while in milk. No attempt was therefore made to determine the minimum protein requirement. Had the object in view been the maintenance of these animals with the minimum protein or at the least cost financially a ration with a larger amount of roughness would have been used. The same grain mixture was employed as was used when the cows were in milk, namely, 4 parts of corn, 2 parts bran, 1 part oilmeal. The proportion used was 1 pound of this grain mixture to 1 pound of alfalfa hay and 4 pounds of silage.

On account of the nature of the investigation in which No. 43 was included she received while on maintenance the ration that has been used in carrying on investigations with growing and fattening steers at this Experiment Station. Her grain mixture consisted of 8 parts of corn to 1 part of oilmeal and 1 pound of alfalfa hay was fed to each 2½ pounds of grain. No. 43 was put on this ration a sufficient length of time before the data on maintenance was taken to adjust her ration to the maintenance point.

The cows were all farrow while these maintenance trials were conducted. The animals were kept nights and during stormy weather in the dairy barn in which the temperature varied considerably. In daytime and during fair weather the animals were turned into a dry lot with no opportunity to secure additional feed. Each animal was weighed daily, in the morning following feeding and before receiving water. The usual difficulties were experienced in maintaining the animals at uniform weight on account of the variations from day to day which is generally assumed to be due to the difference in the contents of the alimentary canal, and to some extent to the amount of water drunk. The average weights by ten days periods are given for each animal in the tables which follow. Apparently there was a gain of about 15 pounds in 160 days with No. 27, and about the same amount with No. 62 in 180 days, with a loss of the same amount for No. 63 in 120 days, a small gain for No. 4 amounting to 14 pounds in 150 days, while No. 43 apparently closed the 130 days trial at the same point at which she began.

The periods as used in the tables are uniformly for ten days. Tables 1, 2, 3, 4, and 5 give the amount of hay, grain, and silage consumed by each animal, also the average weight for each 10 day period. The feedstuffs used were all subjected to chemical analysis, results of which are given in Table 6. In this table the analysis of each different lot of feed is given. By referring to the number given under the heading "Lot" and the tables which follow it is possible to cal-

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culate the amount of each chemical constituent received by each animal as may be desirable.

In calculating the results the composition of the grain mixtures fed is first calculated and then used as one figure. The proportion of the feeds used in the grain mixtures fed at different times is as follows:

FEEDS USED IN THE GRAIN MIXTURES.

	CORN		BRAN		OILMEAL	
	Parts	Lot	Parts	Lot	Parts	Lot
Grain Mixture A.....	4	1	2	1	1	1
Grain Mixture B.....	4	2	2	2	1	1
Grain Mixture C.....	4	1	2	1	1	1
Grain Mixture D....	8	1	0		1	1
Grain Mixture E.....	8	1	0		1	3

Table 7 gives the composition of the grain mixtures derived in the manner explained. Tables 8, 9, 10, 11, and 12 give the composition of the rations fed the individual animals together with the totals and the amount of each constituent received per day.

In making further calculations use is made of the digestion coefficients as given in Table 13, employing the average figures as given by Jordan.[1] The digestion coefficient is calculated for the grain mixtures in order that it may be employed as a single figure.

Tables 14 and 15 give the digestible nutrients in the rations calculated according to the average digestion coefficients as given. The total received per day, and per day per thousand pounds live weight is also included. As was previously stated no attempt was made to determine the minimum protein requirement but rather to determine the proportion of a normal ration that will be used for maintenance.

COMPARISON WITH ARMSBY'S STANDARD.

In order that the maintenance requirement as found for these five cows may be compared with that suggested by Armsby the ration received by each cow is calculated from the tables pre-

[1] Jordan, "Feeding of Farm Animals."

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pared by him[1] and the amount of dry matter, digestible protein and energy value expressed in therms is given, with his maintenance figures for an animal of the same weight below for comparison. These calculations are found in Tables 16 and 17. It will be observed from this data that the maintenance requirements of three of these animals expressed in therms agrees reasonably close with the figures presented by Armsby. Altho there is considerable variation the average for the four receiving the normal ration is 6.08 therms per 1000 pounds as compared with 6.00 as given by Armsby. Cow 43 shows considerable higher requirements. The latter animal, as previously explained, received the ration as ordinarily used for fattening steers. No. 62 and No. 63 both show somewhat less maintenance requirement than called for by Armsby's tables. These two animals as shown by the illustrations carried more flesh in proportion to the size of their frame than did No. 27 or No. 4 which may be of some significance. No. 4 was an animal equal in size as far as skeleton and body is concerned to any of the others but carried considerably less flesh.

Since No. 43 received a ration which is quite different in composition from that usually fed a cow producing milk it is perhaps as well to leave her out in preparing an average.

COMPARISON WITH HAECKER'S STANDARD.

A comparison was also made of the maintenance requirements as found for these five cows as compared with the maintenance ration as given by Haecker.[2] Since a complete statement is not given of the ration the animals received upon which he based his standard it is impossible to make an entirely satisfactory comparison. His ration of maintenance calls for .7 lbs. of digestible protein, 7 lbs. digestible carbohydrates, .1 lb. digestible fat. In order to get the two rations on a comparable basis the energy value is calculated according to Kellner's figures as quoted by Armsby,[3] except the fat is given a value of 2100 C. per pound intermediate between coarse feeds and grains. This is done since Haecker does not state how much of the fat in the ration he used came from the roughness and how much from the grain.

[1]Farmers' Bulletin 346 U. S. Dept. Agric.
[2]Bulletin 79 Minn. Exp. Sta.
[3]Bul. 71 Pa. Exp. Sta. p. 14.

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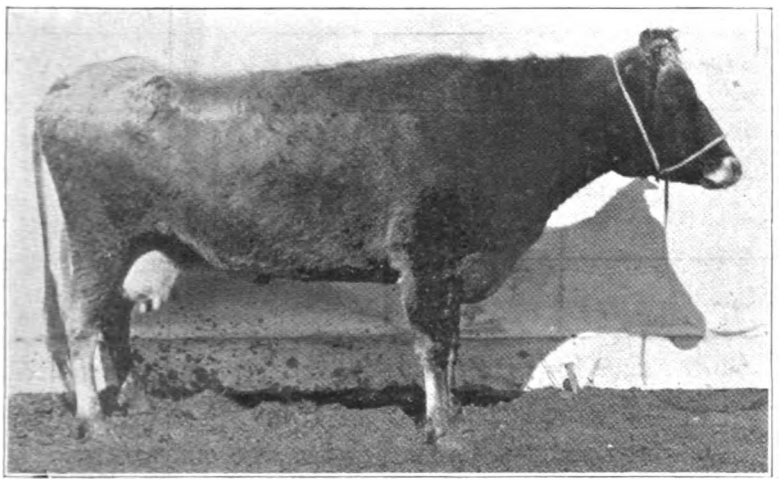

NO. 4. PEDRO'S ALPHEA ELF 168587.
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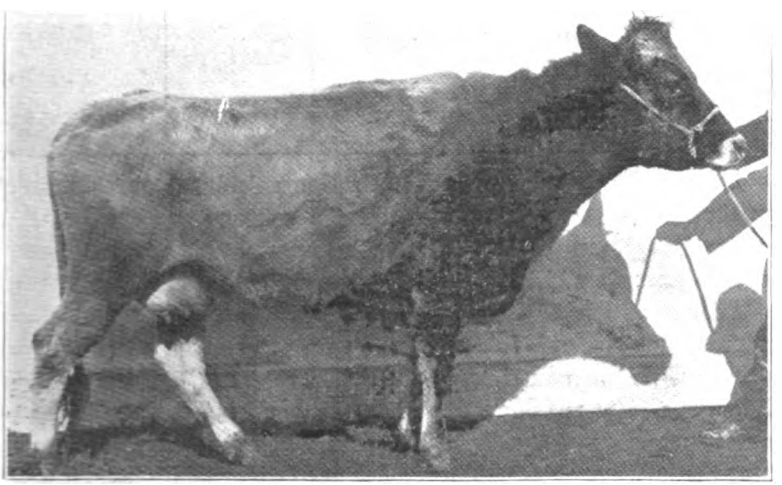

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HAECKER'S MAINTENANCE RATION.

1000 lb. cow.

Digestible Nutrients	lbs.	Energy Value Per lb. in Therms	Total Energy Value in Therms
Protein.....	0.7	1.016	.711
Carbohydrates.....	7.0	1.071	7.497
Fat.....	0.1	2.100	.210
Total			8.418

On the same basis the rations fed the five Jersey cows had the following value:

DIGESTIBLE NUTRIENTS PER DAY PER 1000 LBS. WEIGHT.

	Lbs. Digestible Protein	Lbs. Digestible Carbohydrates	Lbs. Digestible Fat	Energy Value per 100 lbs. weight. Themes
No. 27.....	.949	6.005	.374	8.180
No. 62.....	.839	5.210	.309	7.081
No. 63.....	.868	5.368	.318	7.299
No. 4.....	1.111	7.018	.442	9.573
No. 43.....	1.122	6.993	.492	9.663

The energy value of Haecker's ration calculated on this basis is 8.42 therms per 1000 pounds while the average required to maintain the four Jersey cows receiving the normal dairy ration was 8.61 per 1000 pounds which shows a rather close agreement.

Two digestion trials were conducted with Nos. 27 and 62. In Research Bul. No. 4, Table 13 is shown a comparison of the per cent digested while on maintenance and when receiving full ration. It is pointed out in that connection that the per cent digested when on maintenance is considerably higher than when receiving a full ration. Under the latter conditions the coefficient of digestibility agrees reasonably well with the averages as ordinarily given.

In Table 18 under the heading "Average" is found the amount of the several constituents digested applying the average figures given by Jordan. Under the heading "Actual" is given the amounts digested as found by trial. The average digestibility of the total ration by average digestion coefficients should be 69.1 per cent while No. 27 showed an average of 73.79 and No. 62, 72.19 per cent.

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MAINTENANCE REQUIREMENTS OF NO. 27 AND NO. 62 CALCULATED FROM DIGESTION TRIAL.

Since data is available regarding the chemical composition of the food consumed on maintenance, also a digestion trial for these two animals when on maintenance it is possible to calculate the requirements of these two animals more accurately than by using average figures as is done in Tables 14-17.

In making these calculations the method followed by Armsby[1] in preparing his table of "Production Values" of feed stuffs was used except that the amount to be subtracted for crude fibre in silage was made in accordance with that suggested by Kellner [2] and more recently adopted by Dr. Armsby.[3]

In making these calculations it was assumed that the per cent of each constituent digested varied from average figures[4] in the same ratio that the total amount of that constituent digested according to the digestion trial varied from what would have been digested according to average digestion coefficients. For example in Table 13, Research Bul. No. 4, cow No. 27 is seen to have digested 67.32 per cent of the protein in the ration while according to the average digestion coefficients she should have digested 69.4. That is, the per cent digested was 97.0 per cent of what would be expected from the average figures of digestion. It was therefore assumed that the digestion coefficient for the protein in each food stuff was 97.0 per cent of the average figures. The same plan was followed with each constituent. The amount of amid free protein was calculated by assuming that the same proportion of the digestible protein was in the amid form as was assumed by Dr. Armsby in preparing his table on "Production Values." The value of the ration in therms was calculated by finding the energy value of each constituent of the ration as outlined in Pa. Bulletin No. 71 using the factors for each feed as given by Kellner.[5]

According to this method of calculation the energy value of the ration required for maintenance by the two animals was as follows:

1. Bul. 71 Pa. Exp. Station p. 14.
2. Die Ernahrung der landwirthschaftlichen Nutztiere p. 581.
3. Farmers' Bulletin 346 U. S. Dept. Agric.
4. Average digestion coefficients used are from Jordan's—"The Feeding of Animals."
5. Loc. cit. p 582.

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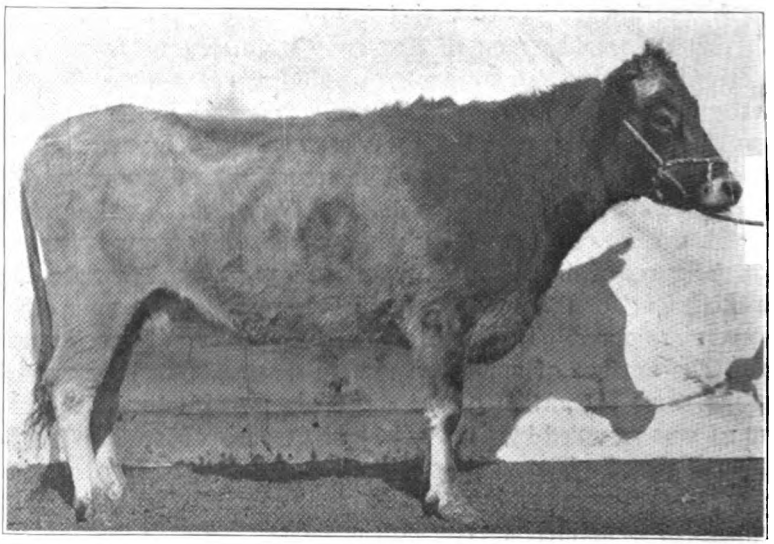
	Live Weight Lbs.	Energy Value Maintenance Ration. Therms.	Armsby's Standard. Therms.
No. 27	890	6.43	5.55
No. 62	917	5.56	5.56

The average for the two is 6.00 therms or about .5 therms higher than the standard suggested by Armsby.[1]

By comparing with Table 16 where the calculations are based upon average figures for both composition and digestion it will be seen that the results are higher in this case when based upon the results of the digestion trial and analysis of the food consumed. This results from the fact that the per cent digested by these two cows was above the average figures.



NO. 63. PEDRO'S GRACE BRIGGS 197836.
Photographed After 4 Months on Maintenance.

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The average energy value of the ration given two cows with which a digestion trial was made that admits of more accurate calculation was 6.00 therms while for animals of this weight the energy value required is estimated at 5.56 therms by Armsby.

The general conclusion from the results with four Jersey cows is that the average maintenance requirements of these animals is quite close to the standards suggested by Armsby and by Haecker.

TABLE 1.

NO. 27.

SUMMARY OF FEED CONSUMED ON MAINTENANCE.

October 30, 1908 to April 7, 1909.

Period	Grain	Alfalfa Hay	Silage	Aver. Weight
1.....	40	40	160	881.1
2.....	37.5	37.5	150	889.5
3.....	33	33	132	887.4
4.....	33	33	132	884.2
5.....	33	33	132	888.9
6.....	32.4	32.4	129.6	895.7
7.....	31	31	124	891.4
8.....	31	31	124	871
9.....	31	33	132	882.4
10.....	32	32	128	888.9
11.....	32	32	128	898.5
12.....	32	32	128	891.1
13.....	32	32	128	902.8
14.....	32	32	128	891.1
15.....	32	32	128	889.4
16.....	32	32	128	896.7
Total.....	525.9	527.9	2111.6	889.4
per day.....	3.29	3.30	13.20	

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TABLE 2.

NO. 62.

SUMMARY OF FEED CONSUMED ON MAINTENANCE.

August 31, 1908 to February 26, 1909.

Period	Grain	Alfalfa Hay	Silage	Aver. Weight
1.....	30	30	123	906
2.....	30	30	120	904.5
3.....	30	30	120	911
4.....	30	30	120	911
5.....	30	30	120	913.5
6.....	31	31	124	904
7.....	30	30	120	908.9
8.....	30	30	120	911.6
9.....	30	30	120	917.4
10.....	30	30	120	921.2
11.....	29.4	29.4	117.6	929.2
12.....	28	28	112	924.6
13.....	28	28	112	923.7
14.....	28	28	112	924.5
15.....	28	28	112	918.3
16.....	28	28	112	922.4
17.....	28	28	112	924.1
18.....	28	28	120	924.4
Total.....	526.4	526.4	2116.6	916.7
Per day.....	2.92	2.92	11.76	

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TABLE 3.

NO. 63.

SUMMARY OF FEED CONSUMED ON MAINTENANCE.

October 20, 1908 to February 16, 1909.

Period	Grain	Alfalfa Hay	Silage	Aver. Weight
1.....	32	32	136	895
2.....	32	32	132	918.6
3.....	30	30	120	878
4.....	30	30	120	891.7
5.....	30	30	120	882.3
6.....	30	30	120	888.4
7.....	29.4	29.4	117.6	900.1
8.....	28	28	112	883.9
9.....	28	28	112	874.5
10.....	28	28	112	882.7
11.....	28	28	112	885.7
12.....	28	28	112	881.8
Total.....	353.4	353.4	1425.6	888.6
Per day.....	2.95	2.95	11.88	

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TABLE 4.

NO. 4.

SUMMARY OF FEED CONSUMED ON MAINTENANCE.

October 20, 1908, to March 18, 1909.

Period	Grain	Alfalfa Hay	Silage	Aver. Weight
1.....	38	38	152	794.5
2.....	36.1	36.1	144.4	819.1
3.....	30	30	120	799.4
4.....	30	30	120	806.4
5.....	30	30	120	792.5
6.....	31	31	124	794.2
7.....	31	31	124	785.6
8.....	31	31	124	788.4
9.....	31	31	124	772.5
10.....	33	33.1	132	766
11.....	35.8	35.8	143.2	768.4
12.....	40	40	160	786
13.....	40	40	160	796.8
14.....	37.3	37.3	158.8	803.4
15.....	39.7	39.7	160	810.7
Total.....	513.9	514	2066.4	792.3
Per day.....	3.43	3.43	13.78	

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TABLE 5.

NO. 43.

SUMMARY OF FEED CONSUMED ON MAINTENANCE.

November 9, 1908, to March 18, 1909.

Period	Grain	Hay	Aver. Weigh
1	67	26.8	733.2
2	70	28	748.4
3	61	24.4	743
4	60	24	736.9
5	60	24	752.4
6	60	24	730
7	60	24	730
8	60	24	734.1
9	60	24	729.3
10	60	24	732.2
11	60	24	728.8
12	66	26.4	728.4
13	79	31.6	735.2
Total	823	329.2	735.5
Per day	6.33	2.53	

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TABLE 6.

CHEMICAL ANALYSIS OF FEED USED IN MAINTENANCE TRIALS.

	Lot	Per cent Dry Matter	Per cent Ash	Per cent Protein	Per cent Crude Fibre	Per cent Nitrogen Free Extract	Per cent Ether Extract
Hay.....	1	95.06	9.03	16.388	28.72	38.202	2.72
Hay.....	2	91.09	7.87	14.75	31.60	34.80	2.07
Hay.....	3	94.21	8.96	14.063	32.99	35.84	2.38
Silage.....	1	39.86	2.539	3.465	10.024	22.404	1.423
Silage.....	2	29.99	1.967	2.432	7.447	17.129	1.017
Silage.....	3	30.19	1.869	1.421	6.999	18.227	1.676
Corn.....	1	90.25	1.50	8.906	1.813	72.804	5.230
Corn.....	2	94.07	1.19	8.844	2.030	78.236	3.770
Bran.....	1	89.82	6.75	14.440	9.190	52.460	6.980
Bran.....	2	89.28	6.293	14.594	10.250	53.503	4.645
Oilmeal.....	1	90.89	5.363	34.363	7.975	36.450	6.736
Oilmeal.....	2	93.45	5.54	34.000	8.03	38.570	7.310
Oilmeal.....	3	91.50	5.385	34.725	8.185	36.270	6.932

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TABLE 7.

COMPOSITION OF THE GRAIN MIXTURES.

Lot	Per cent Dry Matter	Per cent Ash	Per cent Protein	Per cent Crude Fibre	Per cent Nitrogen Free Ext.	Per cent Ether Extract
A	90.22	3.55	14.124	4.798	61.798	5.94
B	92.90	3.555	14.074	5.235	65.504	4.526
C	90.31	3.555	14.175	4.831	61.772	5.973
D	90.32	1.929	11.743	2.508	68.764	5.397
E	90.39	1.932	11.775	2.521	68.744	5.419

TABLE 8.

COMPOSITION OF THE GRAIN MIXTURES.

NO. 27.

Weights in Pounds.

	Lot	Lbs. Fed	Dry Matter	Ash	Protein	Crude Fibre	Nitrogen free Extract	Ether Extract
Alfalfa Hay.	2	527.9	480.86	41.55	77.87	166.82	183.71	10.93
Silage..	2	1215.6	364.56	23.91	29.56	90.53	208.22	12.36
Silage..	3	896.0	270.50	16.75	12.73	62.71	163.31	15.02
Grain..	A	301.9	272.37	10.72	42.64	14.49	186.56	17.93
Grain..	B	32.0	29.73	1.14	4.50	1.68	20.96	1.45
Grain..	C	192.0	173.36	6.83	27.22	9.28	118.60	11.47
Total 160days			1591.38	100.90	194.52	345.51	881.36	69.16
Per day			9.95	.63	1.22	2.16	5.51	.43

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TABLE 9.

COMPOSITION OF RATION FED.

NO. 62.

Weights in Pounds.

	Lot	Lbs. Fed	Dry Matter	Ash	Protein	Crude Fibre	Nitrogen free Extract	Ether Extract
Alfalfa Hay.	1	30.0	28.52	2.71	4.92	8.62	11.46	.82
Alfalfa Hay..	2	468.4	426.67	36.86	69.09	148.01	163.00	9.70
Alfalfa Hay..	3	28.0	26.38	2.51	3.94	9.24	10.04	.67
Silage..	1	123.0	49.03	3.12	4.26	12.33	27.56	1.75
Silage..	2	1649.6	494.72	32.45	40.12	122.85	282.56	16.78
Silage..	3	344.0	103.85	6.43	4.89	24.08	62.70	5.77
Grain..	A	498.4	449.66	17.69	70.39	23.91	307.99	29.60
Grain..	B	28.0	26.01	1.00	3.94	1.47	18.34	1.27
Total 180days	....	.....	1604.84	102.77	201.55	350.51	883.65	66.36
Per day			8.92	.57	1.12	1.95	4.91	.37

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TABLE 10.

COMPOSITION OF RATION FED.

NO. 63.

Weights in Pounds.

	Lot	Lbs. Fed	Dry Matter	Ash	Protein	Crude Fibre	Nitrogen free Extract	Ether Extract
Alfalfa Hay..	2	353.4	321.91	27.81	52.13	111.67	122.98	7.32
Silage..	2	1201.6	360.36	23.64	29.22	89.48	205.82	12.22
Silage..	3	224.0	67.63	4.19	3.18	15.68	40.83	3.75
Grain..	A	353.4	318.84	12.55	49.91	16.96	218.39	20.99
Total 120days	....	.....	1068.74	68.19	134.44	233.79	588.02	44.28
Per day	....	.....	8.22	.52	1.03	1.80	4.52	.34

TABLE 11.

COMPOSITION OF RATION FED.

NO. 4.

Weights in Pounds.

	Lot	Lbs. Fed	Dry Matter	Ash	Protein	Crude Fibre	Nitrogen free Extract	Ether Extract
Alfalfa Hay..	2	514.0	468.20	40.45	75.82	162.42	178.87	10.64
Silage..	2	1284.4	385.19	25.26	31.24	95.65	220.00	13.06
Silage..	3	782.0	236.09	14.62	11.11	54.73	142.54	13.11
Grain..	A	444.6	401.14	15.78	62.80	21.33	274.75	26.41
Grain..	C	69.3	62.59	2.46	9.82	3.35	42.81	4.14
Total 150days	....	.....	1553.31	98.57	190.83	337.48	858.97	67.36
Per day	....	.....	10.36	.66	1.27	2.25	5.73	.45

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TABLE 12.

COMPOSITION OF RATION FED.

NO. 43.

Weights in Pounds.

	Lot	Lbs. Fed	Dry Matter	Ash	Protein	Crude Fibre	Nitrogen free Extract	Ether Extract
Alfalfa Hay..	2	329.2	299.87	25.91	48.56	104.03	114.56	6.81
Grain..	D	690.0	623.21	13.31	81.03	17.31	474.47	37.24
Grain..	E	133.0	120.22	2.57	15.66	3.35	91.43	7.21
Total 130days	....	.....	1043.30	41.79	145.25	124.69	680.46	51.26
Per day	....	.....	8.03	.32	1.12	.96	5.23	.39

TABLE 13.

DIGESTION COEFFICIENTS OF FEEDS USED.

	Protein	Fibre	Nitrogen Free Extract	Ether Extract
Alfalfa Hay.....	72	46	69.2	51
Silage.....	49.3	66.7	68.6	80
Corn.....	67.9	....	94.6	92.1
Bran.....	77.8	28.6	69.4	68
Oilmeal.....	88.8	57	77.6	88.6
Grain Mixture A.....	78.1	37.2	87.1	83.4
Grain Mixture B.....	78.0	36.6	87.3	84.2
Grain Mixture C.....	78.1	37.4	87.1	83.5
Grain Mixture D.	74.7	57.0	93.6	91.6
Grain Mixture E.....	74.8	57.0	93.6	91.6

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TABLE 14.

DIGESTIBLE NUTRIENTS IN RATIONS FED.

By average digestion coefficients.

NO. *27*.

	Dry Matter	Protein	Carbo-hydrates	Ether Extract
Alfalfa Hay.....	480.86	56.07	203.86	5.57
Silage.....	635.06	20.85	357.08	21.90
Grain.....	475.46	58.07	293.55	25.77
Total.....	1591.38	134.99	854.49	53.24
Per day.....	9.95	.844	5.341	.333
Per day per 1000 lbs..	11.183	.949	6.005	.374

No. 62

	Dry Matter	Protein	Carbo-hydrates	Ether Extract
Alfalfa Hay.....	481.57	56.12	203.97	5.71
Silage.....	647.60	24.29	341.98	19.44
Grain.....	475.67	58.05	293.70	25.76
Total.....	1604.83	138.46	859.65	50.89
Per day.....	8.916	.769	4.776	.283
Per day per 1000 lbs..	9.726	.839	5.210	.309

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TABLE 15.

DIGESTIBLE NUTRIENTS IN RATIONS FED.

By average digestion coefficients.

NO 63.

	Dry Matter	Protein	Carbo-Hydrates	Ether Extract
Alfalfa Hay.....	321.91	37.53	136.47	3.73
Silage.....	427.99	15.97	239.34	12.78
Grain.....	318.84	38.98	196.53	17.51
Total.....	1068.74	92.49	572.34	34.02
Per day.....	8.906	.771	4.770	.283
Per day per 1000 lbs..	10.023	.868	5.368	.318

NO. 4.

	Dry Matter	Protein	Carbo-Hydrates	Ether Extract
Alfalfa Hay.....	468.20	54.59	198.49	5.43
Silage.....	621.28	20.89	349.01	20.94
Grain.....	463.73	56.72	285.78	25.48
Total.....	1553.21	132.20	833.28	51.85
Per day.....	10.35	.88	5.56	.35
Per day per 1000 lbs..	13.063	1.111	7.018	.442

NO. 43.

	Dry Matter	Protein	Carbo-Hydrates	Ether Extract
Alfalfa Hay.....	299.87	34.96	127.13	3.47
Grain.....	743.43	72.24	541.46	40.62
Total.....	1043.30	107.20	668.59	44.19
Per day.....	8.025	.825	5.143	.340
Per day per 1000 lbs..	10.911	1.122	6.993	.462

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TABLE 16.

DRY MATTER, DIGESTIBLE PROTEIN, AND ENERGY VALUE CALCULATED FROM ARMSBY'S TABLES.

NO. 27.

	Lbs. Fed	Dry Matter	Digestible Protein	Energy Value Therms
Alfalfa Hay	527.9	483.56	36.58	181.65
Silage	2111.6	540.57	18.58	349.68
Grain	525.9	468.37	56.43	398.74
Total	.....	1492.50	111.59	930.07
Per day	.....	9.33	.70	5.81
Armsby's Standard 890 lbs.	.....	.....	.46	5.55

NO 62.

	Lbs. Fed	Dry Matter	Digestible Protein	Energy Value Therms
Alfalfa Hay	526.4	482.18	36.48	181.13
Silage	2116.6	541.85	18.63	350.51
Grain	526.4	468.81	56.48	399.12
Total	.....	1492.84	111.59	930.76
Per day	.....	8.29	.62	5.17
Armsby's Standard 917 lbs.	.....	.....	.47	5.66

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TABLE 17.

DRY MATTER, DIGESTIBLE PROTEIN, AND ENERGY VALUE CALCULATED FROM ARMSBY'S TABLES.

NO 63.

	Lbs. Fed	Dry Matter	Digestible Protein	Energy Value Therms
Alfalfa Hay..	353.4	323.71	24.49	121.60
Silage.	1425.6	364.95	12.55	236.08
Grain	353.4	314.74	37.93	267.95
Total.		1003.40	74.97	625.63
Per day.		8.36	.62	5.21
Armsby's Standard 890 lbs.			.46	5.55

NO. 4.

	Lbs. Fed	Dry Matter	Digestible Protein	Energy Value Therms
Alfalfa Hay.	514.0	470.82	35.62	176.87
Silage.	2066.4	529.00	18.18	342.20
Grain	513.9	457.68	55.14	389.64
Total.		1457.50	108.94	908.71
Per day.		9.72	.73	6.06
Armsby's Standard 792 lbs.			.43	5.14

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NO. 43.

	Lbs. Fed	Dry Matter	Digestible Protein	Energy Value Therms
Alfalfa Hay.....	329.2	301.55	22.81	113.28
Silage.....	.....	.....	.....	.....
Grain.....	823.0	734.94	74.89	722.10
Total.....	.....	1036.49	97.70	835.38
Per day.....	.....	7.97	.75	6.43
Armsby's Standard 792 lbs.....	.....	.....	.41	4.89

TABLE 18.

NUTRIENTS DIGESTED DURING MAINTENANCE PERIOD, CALCULATED ACCORDING TO AVERAGE AND ACTUAL DIGESTIVE COEFFICIENTS.

	Protein		Crude Fibre		Nitrogen-free Extract		Ether Extract	
	Aver.	Actual	Aver.	Actual	Aver.	Actual	Aver.	Actual
No. 27....	135.00	130.95	182.08	191.17	657.49	723.77	53.25	50.60
No. 62....	138.46	132.10	188.57	182.48	666.27	715.67	50.89	49.05
Per day								
No. 27....	.844	.818	1.138	1.195	4.109	4.524	.333	.316
No. 62....	.769	.734	1.048	1.014	3.702	3.976	.283	.273

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SUMMARY AND CONCLUSIONS.

The maintenance requirement was determined for two cows previously used in digestion trials and for three other high class Jersey cows for periods ranging from 120 to 180 days. Four of these received the same ration except in amount as when in full flow of milk which was, corn silage 4 parts, alfalfa hay 1 part, grain mixture 1 part.

The fifth cow received the ration used at this Experiment Station for fattening steers. Complete chemical analyses were made of all food consumed. No attempt was made to determine the minimum protein requirements. The results are calculated as follows:

Constituents per day consumed, digestible nutrients using average digestion coefficients, digestible protein and energy value calculated by using Armsby's "Production Value" table, and for the two cows with which the digestion trial was made, the energy value after the plan followed by Armsby in preparing his "Production Values" table.

Armsby gives 6.00 therms energy value per 1000 pounds live weight for maintenance. The data presented shows that while there was some variation with the individuals that the four receiving the normal dairy ration averaged 6.08 therms per 1000 pounds calculated by using Armsby's "Production Value" tables.

A comparison was made with Haecker's Standard by reducing it to energy value by using Kellner's "Production Values." Calculated in this way the energy value of Haecker's ration is 8.42 therms for 1000 pounds live weight. Calculated in the same manner the average energy value required by the four cows receiving the normal ration was 8.61 therms.

The average energy value of the ration given two cows, with which a digestion trial was made that admits of more accurate calculation, was 6.00 therms while for animals of this weight the energy value required is estimated at 5.56 therms by Armsby.

The general conclusion from the results with four Jersey cows is that the average maintenance requirements of these animals is quite close to the standard, suggested by Armsby and by Haecker.

1. Farmers' Bul. 346 U. S. Dept. Agric. p. 16.

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The Distribution of Farm Labor

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February, 1913

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AGRICULTURAL EXPERIMENT STATION

The Distribution of Farm Labor

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FOREWORD

The bulletin herewith presented by O. R. Johnson is the first prepared by the Department of Farm Management. The investigational work upon which this report is based was conducted in cooperation with the Bureau of Plant Industry of the United States Department of Agriculture. The problem here considered is one upon which there is very little definite information but is nevertheless one of importance in the economic organization of a farm business. This investigation is typical of the class of investigation which is being followed by the Farm Management Department.

F. B. MUMFORD,
Director.

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THE DISTRIBUTION OF FARM LABOR.

O. R. JOHNSON

Labor as a factor in farm management is of great importance. The success with which this factor is handled, determines to a great extent the success of the business. The labor phase of farming may be broadly divided into two parts: the labor equipment and the labor requirement. Labor equipment includes men and horses kept for the purpose of doing the farm work. Labor requirement includes the labor necessary to carry on farm operations. These two factors are usually intended to be equal in quantity. In other words, a farmer never intends to have more labor available than required, or vice versa. A study of labor distribution is a study of the adjustment of these factors. The problem of procuring efficient labor, commonly spoken of as the "Labor Problem" is not here considered. The adjustment of labor equipment to labor requirement of any farming system is more or less affected by special conditions. The simplest adjustment is found where the manager of the business finds it possible to employ all the labor equipment—both man and horse—for as short or as long a time as is necessary. From this simple process, the adjustment problem goes to the other extreme, where a manager must employ his help for the entire year in order to obtain help which is efficient and dependable. For the latter case the task of adjusting the two factors is most difficult. With the increasing difficulty of obtaining labor equipment goes the increasing difficulty of adjusting the two factors under any conditions. Nearly all managers have learned through long experience which farm enterprises give them the most satisfactory results under their conditions. If conditions have caused them to adopt a sytsem of farming which gives a fairly regular amount of labor through the year, this also lessens the difficulty of adjusting the two factors. Such a state of affairs however is rarely found in Missouri. The more general condition includes very irregular labor requirements together with the almost impossibility of obtaining extra, short time, labor equipment. Under ideal conditions, the meeting of this last situation will demand that the labor requirements be so influenced that regular employment, of the right kind, can be furnished the hired help during the whole period that help is hired. In the case of horse labor, the horses should be regularly employed all the time they are kept on the farm. It is inquired if some kinds of operations may not be so "very profitable" that the operator can afford to let his labor equipment be idle at some time, because of excessive profit at others. This can be answered by stating that every day labor equipment is idle in-

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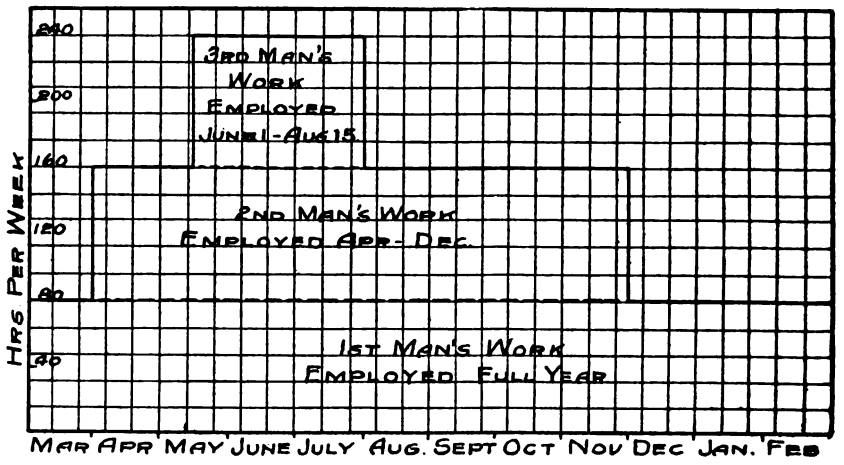
creases the cost of the actual labor done; that if some effort had been made to obtain employment for labor equipment during those idle days, the cost of labor on the "very profitable" operations would have been lessened by the amount of extra employment furnished, thus making the "very profitable" operations more profitable. Thus it is seen that the more regular the "production" labor, the more profitable will be the labor equipment. So it is in the interest of the men who must employ regular labor equipment, with very irregular labor requirements, that this work has been carried on. The men so situated find that, if they are to operate their farms for the year, they must hire enough man labor to do this at the opening of the crop season and keep that labor until the close, or often until the close of the calendar year. These men find it so difficult to employ extra day labor that they must make their plans in such a way that the extra help can, by putting in extra long days in the busy season, handle the work. These men usually find days when there is little to do, as well as days when each man is using every hour of daylight.

So while special conditions make special phases of the problem, there is one thing which all must face; that is, to provide regular work, of the right kind, for their helpers—no matter whether they are hired for a week, a month, or a year; if they employ one man or five at any one time, each man must have work enough to keep

FIGURE 1.—THEORETICAL CONDITION OF EMPLOYMENT FOR MAXIMUM EFFICIENCY.



How labor would appear under ideal conditions for maximum efficiency, each workman employed working regular time per week for his period of employment.

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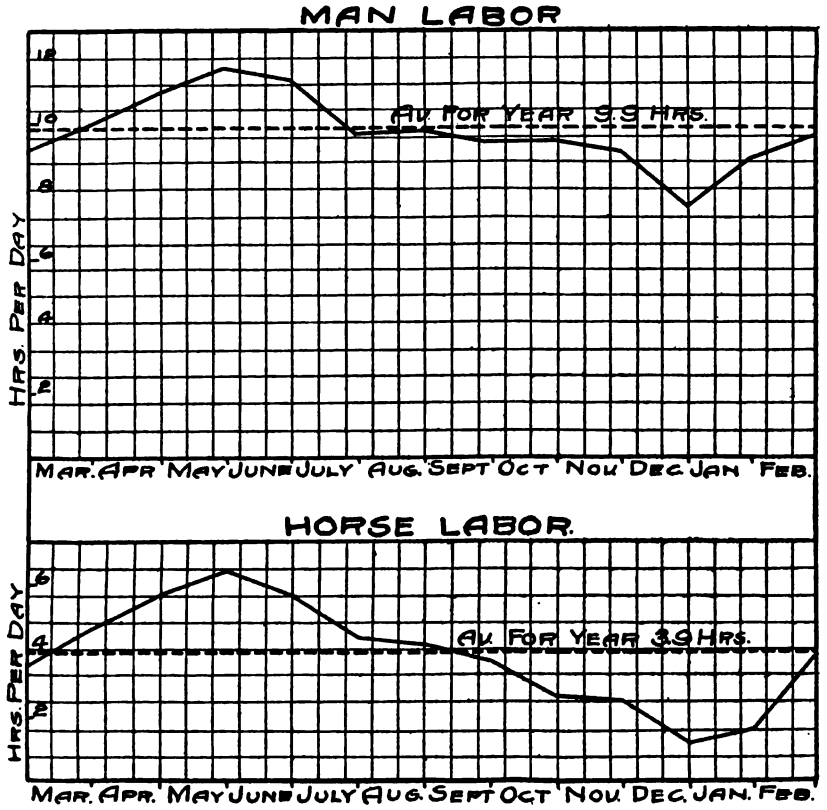
him busy each week he works. Thus labor conditions should appear as shown in the following diagram. Each horizontal line above the base represents the total of each additional workman's labor. This line would appear horizontally if each man were kept busy every day.

This is the condition which exists in other enterprises than farming where the margin of production is very much narrower than in agriculture, so that we can see with the increase of farm efficiency will come the tendency to keep men busy regularly. For the man situated as the average Missouri farmer is situated, it has become almost a necessity that he employ a regular workman or two for at least six months and often for a year. This is in order to be sure of help when he needs it; and hiring a man for the year also enables him to get much more reliable help. In either case, the proprietor

FIGURE 2.—LENGTH OF WORK DAY.



The average length of day worked by men and horses the different months in the year is a variable quantity, emphasizing the need of efforts looking toward more regular employment.

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finds many times when he is not able to give his men as full days of labor as he would like. Also a part of the labor which he furnishes is not of the most productive type. The problem is to furnish regular labor and the right kind of labor.

The problem with horse labor is slightly different and a more difficult one to meet. A manager can usually hire men at some price to help in critical periods, but he finds himself compelled to own horses enough to do the work in the busy season. Consequently when the season of less demand comes he finds it impossible to employ all of his work stock. This is made very evident when we consider the variation in the length of day work per work horse on the farm, as shown by investigations carried on in Missouri by the Farm Management Department, University of Missouri, in co-operation with the Office of Farm Management, United States Department of Agriculture (See Figure 1).

The problem of the management of horse labor is a more limited one than the management of man labor. This is because the two factors in horse labor management, namely, horse equipment and available work, are but little influenced by management at the present time. The present system of diversified farming gives very little labor for the work horse outside the crop season, and when this is remembered, it is not difficult to understand why the greater part of farm work stock equipment boards with the farmer from December to April and through a part of July, August and September. Compared with this conditon, the problem of the management of man labor is a simple one to handle. Man labor equipment is easily reduced or increased as compared with horse labor equipment, and employment for men also much more easily supplied. Because of the fact that on the diversified farm about 60% of a horse's work is put in on field crops, a careful study of the crop situation will help materially in handling horse labor. And when we consider that about 70% of a man's time is devoted to miscellaneous farm labor, it must be admitted that where field labor is arranged in the best possible manner, we will still have to turn to the miscellaneous class of labor to entirely solve the problem in the handling of men. Thus both field and miscellaneous labor must be dealt with in handling this problem; and as field labor requirements are, under the best of conditions, definitely limited by those conditions, this should be provided for first in the best way possible. Then the more flexible miscellaneous labor can be moulded to fill up, as much as possible the periods of less demand during the season. In order to intelligently study any farm problem, you must first make yourself acquainted

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with the actual conditions before any effort can be made to remedy those conditions. So to become familiar with the exact existing conditions with regard to farm labor, an investigation was begun in 1910, on some common types of farms to determine these conditions. Accurate record was made over a period of two years of every hour of labor put in on the farm. This labor has been classified and put in condition to study. This classification is based on the kind of labor performed, and in order to understand the classification, some explanation of the terms used will be necessary.

It will be noticed from the classification (Figure 3) that farm labor has been divided into two classes, namely up-keep, or maintenance, and production. While it is recognized that up-keep or maintenance labor is productive labor, it is separated from the other class of productive labor as indicated, that the difference between the two classes of labor may be more easily seen. The desire to distinguish between these two classes of labor arises from the fact that if a farmer should put in all his labor in maintaining or keeping up his farm and give no time to the other class of production, he would never be ahead at the end of the year's work. Maintenance labor is labor performed in maintaining the farm as a producing machine, or the keeping up of the efficiency of that machine. The farm is looked upon as a producing machine, or factory, and all work consumed in the maintaining of a certain standard of efficiency in that machine is maintenance labor. Production labor, as it is here referred to, includes all labor other than maintenance.

Under up-keep or maintenance labor, we have several divisions. The first is maintenance of equipment, or work of caring for and repairing the farm machinery. The second is maintenace of real estate, and includes repairs of buildings and fences, the prevention of washing, etc. The keeping up of the farm home, or tenant houses, in so far as they are an essential part of the farm by furnishing board for the workmen, is a part of the maintenance division. Labor in caring for and feeding work stock, because they are an essential part of farm equipment, is considered maintenance labor. Also labor chargeable to the personal account of the manager is included in the up-keep division, because such labor is a part of the price for managership.

Now to turn to production labor. The first outstanding class is crop labor, both production and marketing of crops. The other production labor includes six different classes of labor. First, we have the improving of real estate which includes clearing and draining the land, putting up new fences, buildings, water-systems, etc.

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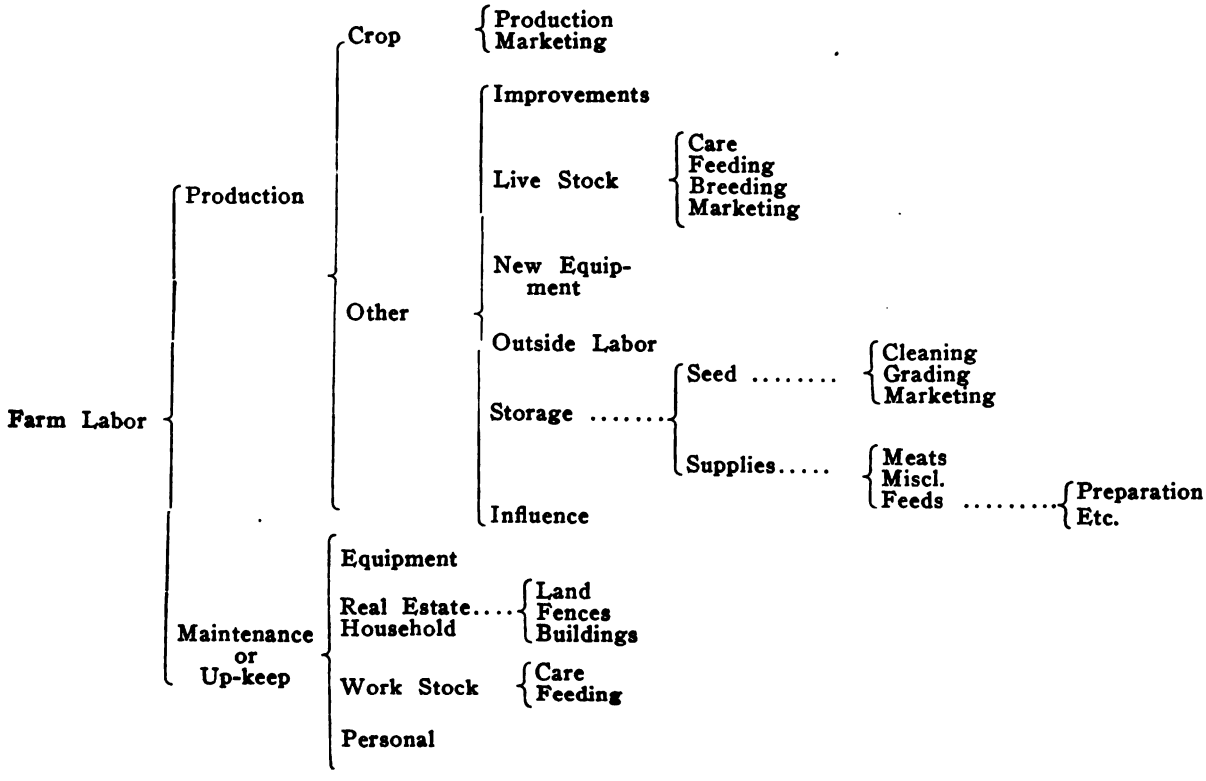
FIGURE 8.—LABOR CLASSIFICATION.



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Then we have the care and feeding of live-stock other than work stock on the farm. This class considers work on all breeding stock, and regular daily feeding and care of all stock, except that time legitimately chargeable to work stock equipment. Here should be mentioned the distinction between work stock and brood mares used as work stock. All regular work of feeding and care of brood mares as work stock has been considered under work stock. Such work as care of a mare at foaling time, taking her to the stallion and all other attention given her that she would not receive as a work mare would be considered in the other live stock class. This account includes the production and marketing of the different classes of live-stock. This factor is either large or small, depending on the importance of live stock on the particular farm. Labor on new equipment included in this division is usually a small factor. Under this class would come only the work of purchasing, bringing home and setting up of new farm machinery. Under outside labor would fall all work done off the farm. This class is of varying importance. It can often be taken advantage of to give men and teams work in idle seasons, thus helping to solve the "keep busy" problem. It is applicable, however, to special conditions in the main. The storage factor is one that can usually be influenced by the manager of the business. The terms used are self-explanatory. A better grade of seed may be produced. It may have more attention, such as cleaning, grading and treating, thus making it more salable. Meat can often be cured on the farm and sold out to a local trade. Special care can be given fruits and vegetables to make them more valuable and provide a larger salable supply. Often extra work can profitably be given to the better preparation of feeds. Also labor performed in obtaining and maintaining good will, under the class called "influence," is included in this division. This class may be either large or small depending on conditions.

Thus we have separated for this study the labor of production from the labor of maintenance on the farm as a machine, because while the labor of maintenance must be performed, the man's profits are measured by his production labor.

In applying the classification of farm labor to find out just what conditions do obtain, the labor records collected from four Missouri farmers were used. In order to conveniently make this study an arbitrary period of time must be taken as the labor unit. Labor in this discussion is always spoken of in terms of hours, man hours and horse hours. A "man hour" is the work of one man for one hour, and by horse hour is meant the work of one horse for one hour.

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Thus if a man plows ground in a certain field for four hours and uses three horses, it would be spoken of as four man hours and twelve horse hours. As the hour is a more definite period than the farm day and more convenient, it is used as the labor unit instead of the indefinite day period. In showing the proportion of labor given to the different classses of farm work, the week has been selected as the unit seasonal period. The day would be too short a period to be of value and the month would not give detail enough; as the week contains, uniformly, six work days, it is believed to be a more just and convenient unit of time. In the study of these three classes, labor will therefore be referred to in **hours per week.** The relative amount of each kind of work put in for every week covered by the record is shown. The fact should be here noted that the number of sources of data for the accompanying tables and illustrations are small, and thus the generality of conclusions will be lessened. But the value of the indications brought out are unimpaired and the usefulness of the derived tables is limited only by the small number of sources.

In all cases, any factors apart from the ordinary have been closely studied and all data that would tend to create inaccuracies, because of these extraordinary conditions, have been omitted. In other words, only regular farm enterprises have been included. For convenience of reference the different farms are numbered, and will always be referred to by their special number.

Farm No. 1 is a diversified farm of 103 acres, located in Cooper county. This is a rough farm with some bottom land. A stream runs through part of the farm and each season proves very troublesome because of washout of fences and overflowing. The effect of repairing these fences can be clearly seen in the maintenance curve at different points. This farm is equipped about as the average farm, with medium grade of work stock.

Farm No. 2 is a rolling Northeast Missouri farm in Scotland county; 160 acres, practically all tillable, are devoted to diversified farming. The land would not be considered rough. The equipment is practically all new. Two teams of mules and one team of draft mares are kept on this farm, an extraordinary quality of work stock. The greatest difficulty here from the standpoint of profit is the poor condition of the soil. This does not affect to any great extent the labor situation. In a general way, more work is expended on the crops per acre, but otherwise no influences are found beyond those which influence any labor situation.

Farm No. 3 is a level prairie farm in Boone county. It contains

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160 acres of relatively low productive capacity. General farming is carried on with some attention paid to dairying. Very little equipment is kept on this farm. One of the greatest handcaps of the year's management has been lack of farm equipment. The work stock equipment compares fairly well with Farm No. 1—in other

TABLE I.—COMPARISON OF EQUIPMENT ON FARMS.

Farm	Total Acres	Acres Culti-vated	Acres Per Head Work Stock	Acres Per $100 Equipment	Profit Per Acre	Type of Farming
1	103	73.7	20.6	20.00	.38	Diversified
2	160	107.9	26.6	31.35	2.82	Diversified
3	160	88.0	26.6	80.00	2.68	Div. with dairying on small scale
4	300	136.5	42.8	11.32	3.78	Dairy

TABLE II.—LABOR ON WORK STOCK AND COWS.

Showing the percent of total labor given to work stock and cows on the four farms; also the labor cost of keep per horse per day and per cow per day.

Farm	Total Labor Percent Time to work stock	Total Labor Percent Time to cows	*Cost per Day per horse	*Cost per Day per cow
1	4.50	3.29	$ .038	$ .069
2	7.36	3.34	.057	.156
3	5.16	20.60	.041	.142
4	5.25	20.07	.056	.113

*Charging 15c per hour for man labor and 10c per hour for horse labor.

words, it would be classed as about average. With this farm, as with Farm 2, the soil has been mistreated so that more work is required than on better handled land. This factor is not a large one however. It should be said here, in justice to the operator of Farm 3, that the soil is so low in fertility that under the best of conditions it would be impossible to make any profit on most field crops. Land that will only grow 20 bushels of corn will very seldom return any profit. This farm is the only one not owned in part or wholly by the operator. A rented farm often places a good manager at a disadvantage, through the impossibility of altering or adding improvements to increase convenience and efficiency. This was especially true on Farm 3.

Farm No. 4 is a 300 acre Missouri River hill farm, located in Franklin county. It is a dairy farm primarily, and its soil is in excellent condition. This farm has more equipment than will usually be found on farms; it's work stock equipment is perhaps above the average for quality. The work is mostly done with geldings. The figures for this farm are for 1911, a dry year, and all through the season more work per acre was put in on crops than on the other

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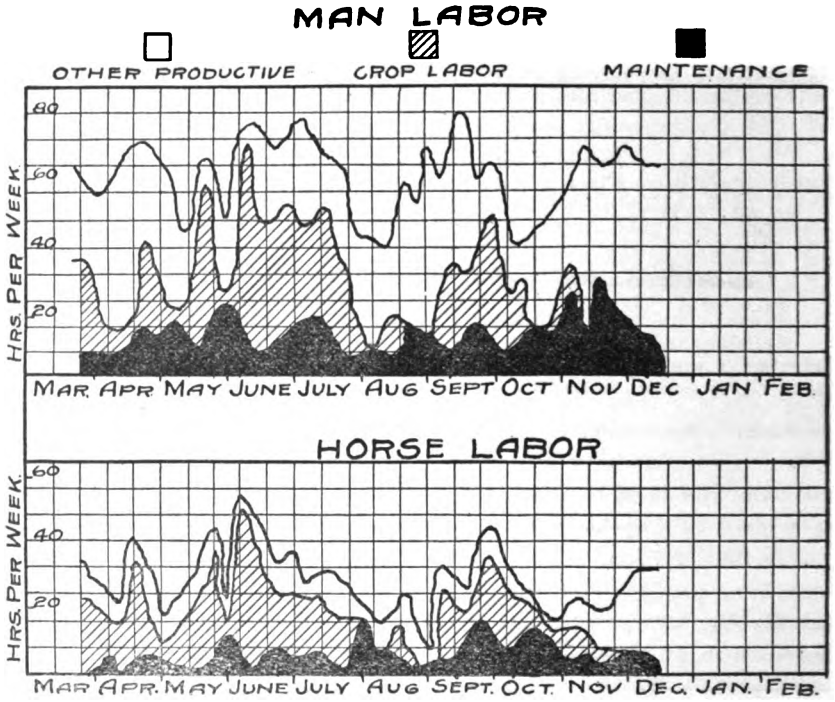
farms, but this will not affect materially the labor distribution.

In a general way, with regard to these four farms, we might say that the productive capacity of the farms would be fairly well represented by the rating of twenty bushels of corn per acre for Farm 3, thirty bushels for Farm 2, forty for Farm 1 and fifty for Farm 4. Comparison of equipment, work stock and machinery, is shown in Table I. With regard to work stock an advantage is seen in the larger farm. While more horse hours' labor were given to each acre on Farm 4, yet the number of horses kept per acre was much smaller. This is emphasized when you consider that the horses on Farm 4 worked an average of approximately 5 hours per

FIGURE 4.—LABOR DISTRIBUTION ON FARM 1.



Showing the distribution of labor per man and per horse each week on Farm 1. A very irregular labor arrangement which does not seem justifiable. The profits per acre were undoubtedly largely affected by this factor.

day, while the average for all the farms was 3.9 hours, or 20% less. This increase in length of day worked was not due so much to the size of farm as it was to the type of farming carried on. The investment in farm machinery in this case does not represent the re-

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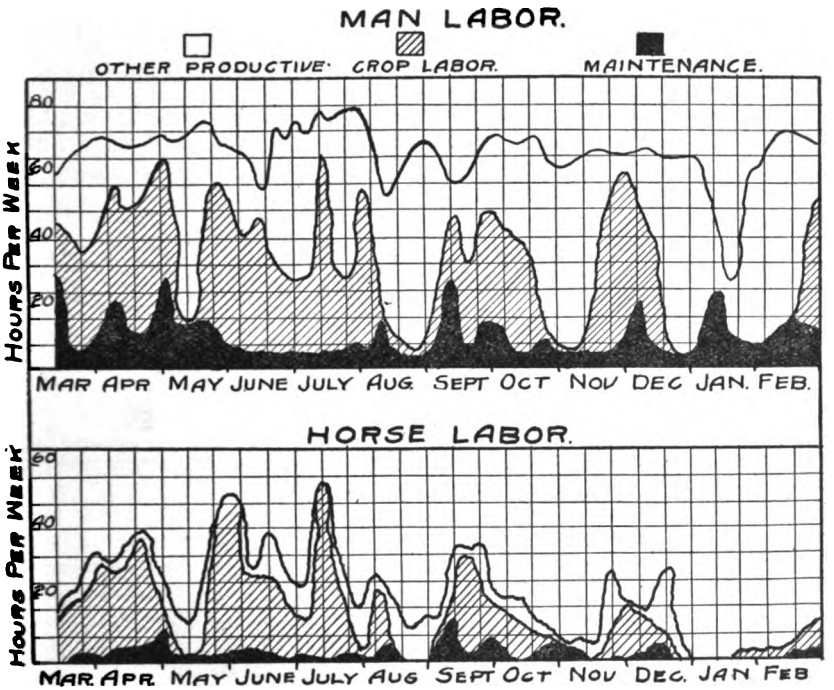
FIGURE 5.—LABOR DISTRIBUTION ON FARM 2.



Showing the proportion of one man's and one horse's time given to the different classes of labor each week on Farm 2. Fair regularity of the total labor curve is seen with man labor, while the horse labor curve is exceedingly irregular. While profits per acre on this farm were slightly more than on Farm 3, this could very easily have been due the better equipment and soil.

lation of investment to size of farm, but rather shows the comparative adequacy of machinery equipment.

With this brief review of the farms, a study of the curves representing the distribution of labor on these farms will prove an interesting one. The labor is figured on the one man and the one horse basis, so the different curves should be comparable.

Farm 1 shows a conspicuous absence of consistent effort to supply labor for men when they were not employed in field work. The second week in May and the end of that month, show a large decrease in labor done, due to rains. Had the work been more carefully planned, other labor would have been supplied at this time. The extreme irregularity of the maintenance labor also shows lack of system in the distribution of labor through the season. Maintenance labor can be controlled in any case to the extent that most work, except care of work stock, will come on days when other

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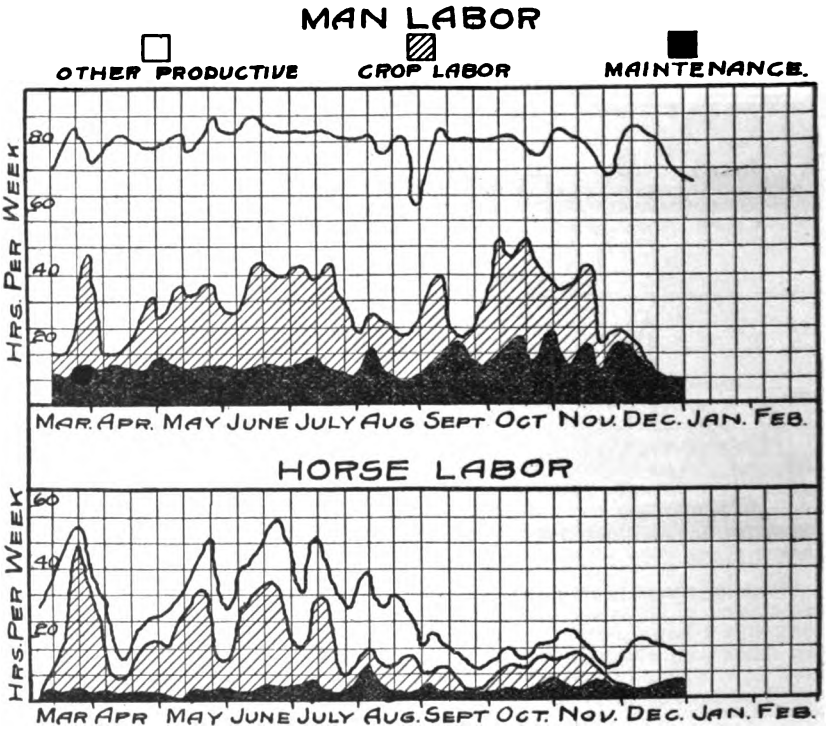
work is scarce. This can be taken advantage of in the fall, winter or early spring, and brings up the labor curve at those times. Farm 1 shows very little effort in that direction. Regarding horse labor on this farm, great irregularity proved to be the rule. Very uneven handling of labor of maintenance together with great dependence on field work for employment is also noticed. The distribution here is a fair representation of the situation on a large number of farms.

FIGURE 6.—LABOR DISTRIBUTION ON FARM 3.



Showing the proportion of one man's or one horse's time given to the three classes of labor each week in the year on Farm 3. More regularity is seen than on any of the farms yet studied. Other production labor here aids in a remarkable way.

Plenty of work in seasons of tillage and harvest, with little in winter, late summer and early fall, is a characteristic of the more common type of farming today.

Farm 2 shows a very successful effort in providing work other than field labor, and balances up the total labor through the year fairly well. One week in January when the work was low is due to

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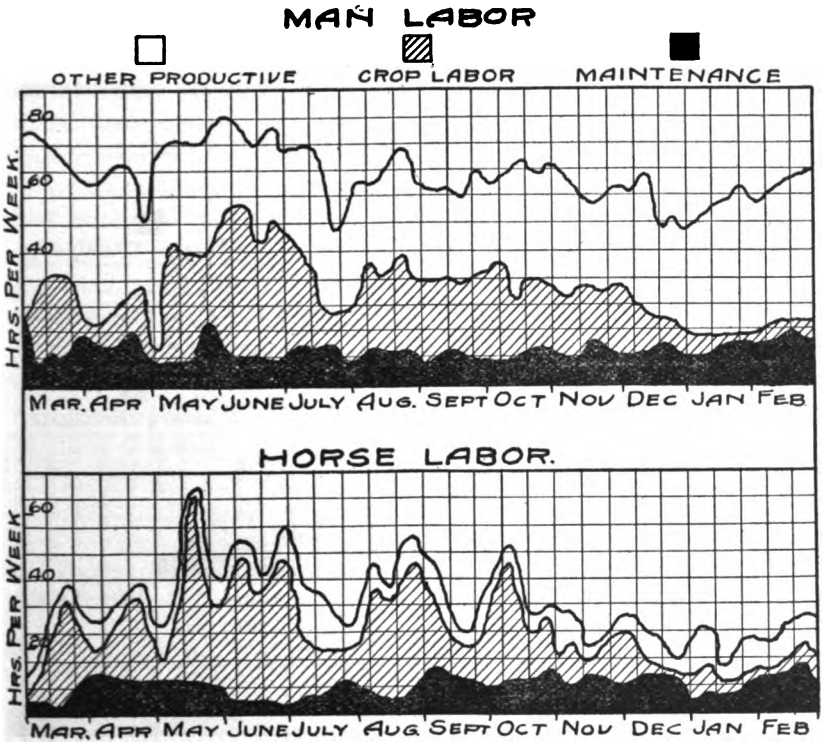
a hard storm, which made it nearly impossible to work at all. The field work on this farm appears extremely irregular, which would

FIGURE 7.—LABOR DISTRIBUTION ON FARM 4.



Showing the proportion of one man's or one horse's time given to the three classes of labor each week in the year on Farm 4. Other production labor plays a large part in the situation. Usually on a home farm with no hired labor less effort is made to supply regular employment than on a rented farm with all hired labor. This comparison holds for Farms 3 and 4. Farm 4 succeeded in giving horses more work each week than any other farm.

indicate that the best cropping system has not been adopted as yet. This is better understood when you consider that this farm was very dilapidated as to soil conditions and improvements. New buildings and fences were being put up at this time, thus making it easy to suppy work out of field hours. Such work can be supplied in quantity on any farm for only a limited time, but when this work is over the total labor curve will take a much different form, unless the other work of the farm is much better developed.

The situation here as regards horse labor is much less satisfactory than on the farm just studied. With a much better class

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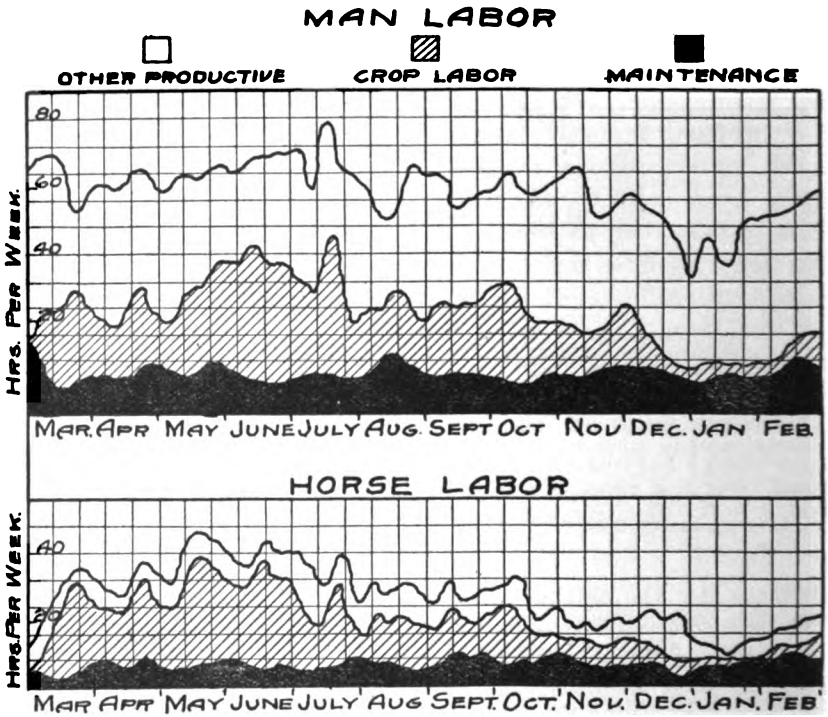
of work stock, the work was more irregular and gave very little evidence of management. It is out of the question to expect a team which does 50 hours' work for a week or two, and then runs free for the next two weeks, to remain in good condition and as able to work as one which works regularly. It will be observed here also that very little work was done outside of field labor. The first half of January the horses were not worked at all. The average length of day worked on this farm was less than on any of the others.

Farms 3 and 4 are very similar as to labor management. More success here is probably due to the dairy factor. The total labor curve on Farm 3 is more regular than that of Farm 4. The fact

FIGURE 8.—AVERAGE LABOR SITUATION OF ALL FARMS.



Showing the proportion of time of one man and one horse given to the three classes of labor each week in the year (Average of all farms).

that Farm 3 was a rented farm with nothing but hired labor, while Farm 4 was a home farm with all work done by father and sons will, in a large measure, explain such a difference. Not so marked an

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effort is made to supply regular work on the home farm usually, as is the case on a farm where all hired labor is employed.

The decrease in labor on Farm 4 early in May was due to a heavy rain which almost suspended all field operations for the week. It is interesting to note that the total curve did not act entirely as the crop curve did, but recovered more quickly. The larger falling off of labor in July was due to the dry weather, the ground becoming so dry that it could not be plowed, and so field work decreased very markedly. The intense cropping system used on this farm makes field work abundant practically all season. More horse labor was furnished at all times on this farm than on Farm 3. The regularity of maintenance labor on Farm 4 is more marked than on any of the other farms.

The horse labor on Farm 3, unlike the rest, shows fairly regular other production labor. This was due largely to the delivering of milk at retail which took enough time to be noticeable. The field labor was a little more regular than on Farms 1 and 2. So also is the maintenance.

On Farm 4 appears the more regular use of horse labor in field work giving perhaps, as much regularity in this respect as will often be found. Other production labor played a fairly regular part in this labor distribution. Maintenance labor is perhaps more regularly handled here than on the other farms. As has been before mentioned, more labor was furnished for the horse equipment on this farm than on the others. The cropping system here was especially adapted to furnishing abundant horse labor. As many as three crops have been harvested from the same acre in a season. The dairy feature of this farm made such a practice possible.

From the study of the horse labor curves we see that much less regularity is shown than in the case of man labor. More regularity of employment will doubtless prove profitable in many cases.

Thus it is seen that distribution of labor on these farms is not ideal. While the dairy farms have a condition much better than the diversified farms, yet extensive improvements must be made there also. While it is possible to change cropping systems slightly, so as to give somewhat more satisfactory results, one will soon be compelled to depend on the other production labor. This is more easily understood when we consider that both other classes of labor are determined by definite factors. Take for instance maintenance labor:—on any farm there are a definite number of work stock, so many buildings and fences, so much machinery, and a certain number of acres of land to maintain. For these different definite quan-

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tities so much of man's and horses' time will be required. A definite amount of time is required to care for work stock or machinery. Thus, so far as total labor goes, with maintenance, the amount is definitely fixed. Now consider crop labor. Every man has his special conditions. Under those conditions he can grow best only a few crops; and because the size of his tillable ground or work force is limited, he will grow only certain acres of each crop. Every acre of each crop grown can receive only a certain number of hours labor. Beyond this limit the increased product on that acre will not pay for the labor, so he stops there. This work can be done only at certain seasons. For instance, in a certain section wheat must be planted between September 10th and November 1st. It must be harvested in June. Such work can not be postponed to a less busy season. So that the whole field here with regard to crop labor is nearly as definitely fixed as that of maintenance labor. This will be found to be the condition of affairs in actual practice.

With this general view of the labor situation, attention is directed to its general phases. The study of special kinds of work will be divided into three classes: crop, other production and maintenance.

TABLE III.—THE PERCENTAGE OF LABOR DISTRIBUTION.

A. MAN LABOR

	Maintenance	Crop	Other Production
Farm 1	20.092	30.112	49.796
Farm 2	15.456	34.218	50.310
Farm 3	14.380	22.960	62.660
Farm 4	16.916	28.140	54.937

B. HORSE LABOR

	Maintenance	Crop	Other Production
Farm 1	21.62	51.60	26.78
Farm 2	6.83	69.48	23.69
Farm 3	6.91	51.48	41.61
Farm 4	20.17	60.10	19.73

Giving the per cent of total man and horse labor devoted to the three classes of labor on each of the four farms.

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TABLE IV.—MONTHLY DISTRIBUTION OF LABOR.

Month	Average hours worked per month		Per cent of time given to crops		Hours given to crop production	
	Man	Horse	Man	Horse	Man	Horse
March	260.9	94.4	31.2	65.3	81.3	61.6
April	277.5	119.0	33.9	63.7	94.2	75.8
May	304.0	155.3	33.3	70.7	101.3	110.0
June	318.0	161.2	47.3	76.0	150.5	122.7
July	317.0	152.2	34.2	60.2	108.3	91.6
August	276.5	118.7	32.3	56.1	89.3	66.5
Sept.	271.5	106.3	31.4	58.2	85.3	61.8
Oct.	265.5	100.2	34.0	58.3	90.2	58.4
Nov.	260.7	67.5	21.5	34.8	56.0	23.5
Dec.	255.2	64.9	15.6	22.6	39.8	14.7
Jan.	225.3	35.3	7.2	19.5	16.2	6.9
Feb.	239.2	41.6	7.8	29.9	18.6	12.4
Totals	3272.3	1216.6	28.4	58.02	931.0	705.9

Giving the average number of hours that man and horse work each month; the per cent of this time devoted to field crops and the equivalent of that percent in hours; showing that men worked over 3000 hours per year and horses over 1200. 28.4% of man's time and 58% of horses' time given to field crops.

CROP LABOR.

The amount of time spent on crops shows in the case of man labor 28% and in the case of horse labor 58%. The hours given to crop work each month varied for man labor from 16 hours in January to 150 hours in June, per man;—and per horse, from 7 hours in January to 122 hours in June (Tables 3 and 4).

The study of crop and rotation requirements shown by the practices on these farms will illustrate the extent to which labor distribution can be influenced by changes of rotation. In studying the amount of labor put in on the different crops on these farms, the month was used as the unit of time. This was done because few crops are produced that must be planted or harvested in any one week. Most operations can be done a little earlier or a little later, than any special time that might be set, so that a smaller division than the month would be of no special value. It was found from the records of the four farms that the number of hours a workman works per month varies from 225 hours in January to 318 hours in June. The percent of this time devoted to field work varies from 7% in January to 47% in June. Similar figures for horse labor have also been determined. Table 4 shows how much labor one man or one horse does on field crops each month in the year.

A very careful study of these variations, and consideration of the fact that these were obtained from actual farm operations will show that such things as seasonal duty, the effect of storms and

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other ordinary delays in farm work, have all been allowed for in these figures so that they represent the actual proportion of time given to crop production. The figures are not estimates, nor are they theoretical. When a man did not work for any reason, his time was not counted. If a rain kept him from the field, if he was sick, if he broke a machine and had to wait for repairs, if the hogs got into the corn field and he had to put them out, the daily record which he made showed that fact. So the figures are not based on the theory that there are 45 days in which ground can be prepared and corn planted, and that 15 of these days will be unavailable, therefore the work must be done in 30 days. Neither are they concerned with the fact that a 14-in plow going at a certain rate for a certain number of hours will turn so much ground, therefore a man will plow so much ground in a certain number of days. The work was done and the time of doing recorded without considering the theoretical results, which might be figured out on paper.

The actual labor requirements of the different farm crops have also been computed from the records of these farms (Tables 5 and 6). These figures show the man hours and horse hours per acre devoted to a particular crop that particular month. So by multiplying the acreage of a crop by the hour requirements per acre that month, one may determine the approximate amount of time which will be required for a crop in any month. This makes possible a study of the degree to which crops conflict from the standpoint of labor; also it will show when more labor will be needed and at about what time it will be needed. This enables one to plan a rotation with a minimum of conflicting operations, and to anticipate the labor requirements for the different months in the year. Such a system carefully worked out would make it possible for a man to avoid having more work than his work force at that time could do. Looking at those results briefly (Table 5 and 6) it will be found that in June, the month that the heaviest work comes with corn, clover also requires a great deal of work, thus illustrating why corn and clover do not fit well together in a rotation from a standpoint of labor requirements. Similar reasoning shows why corn and oats are a popular combination. This data may be directly useful to the man who is confronted with the problem of how much labor to hire and when he will need this labor, or how much work stock he will need to care for a certain number of acres of certain crops. These figures have been reduced to man and horse unit requirements of the common crops for the different months of the year, based on the per cent of time per man and horse given to crop

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work and the hour requirements of the different crops (Tables 4 and 5). Man unit means the available time of a man for field work. Thus 1.5 man units would mean that 1.5 times what one man could give to crop work would be required. The same terms apply to horse units. The man and horse unit requirements for these crops have been worked out for acreages from 1 to 20. Assuming that these results are representative, it will then be possible to determine the number of men and horses required for different rotations each month in the year, or to determine the acreage which can best be handled by a certain amount of man and horse labor available, giving them as regular employment as possible (Tables 7 and 8).

For instance, what would be the requirements for 15 acres of corn for the month of June? Turn to Table 7 which gives the man unit requirements of crops. The first crop we come to in this table is corn. Now follow down the left hand column, which contains acres, to 15. This is the desired acreage. Now move to the right four columns. The fourth one, not counting the acres column, we find contains the number .6705. If we follow up this column, we find it to be headed June. Thus we have by reading in the June column on the same line as the required acreage, the desired man unit requirements. Similar calculation in Table 8 will give horse unit requirements. This is found to be 1.3350, or one and one-third horse's work for June. The same calculation can be made for any acreage for any month of the crops for which material has become available. In making these determinations only the normal handling of crops has been considered. The figures from these tables are taken from the four farms, and because of this fact the best that can be done in balancing up a rotation is to equal the average conditions on the four farms. It simply means that one is relying on those average conditions in using the figures in these tables. That is a safe basis for figuring. One should endeavor to do a little better than those figures indicate, however. Now to follow through a rotation and see how it fits those conditions. Take a simple cropping system of corn, oats, wheat and clover, 20 acres each, determining the labor requirements of that rotation. From the tables of man unit requirements it is found that 20 acres of corn in March requires .036 man units, oats .718 man units, with no man unit requirements for wheat or clover for this month. The total man unit requirement for this rotation for March then would be the sum of requirements for corn and oats, or .754 man units. In other words it would take about three-fourths of the time one man has to devote to crop work to care for such a rotation during

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the month of March. It should be remembered that allowance has already been made for all miscellaneous labor. For horse labor units, by following the same course it is found that 2.158 horse units are required in March. The requirements for the rest of the year determined in the same way, would make the total labor requirements for the year as follows:

Month	Man Units	Horse Units
March	.754	2.158
April	.7680	2.284
May	1.400	2.846
June	1.938	3.044
July	2.582	2.076
August	1.306	1.876
September	2.012	2.508
October	1.292	3.102
November	1.214	1.932
December	1.282	2.190
January	.680	.812
February	.516	.160

Odd acreages of any crop or cropping system can be conveniently determined in the same manner. The acreages larger than 20 may be determined by adding together the units representing complements of that acreage. To illustrate: if there are 35 acres of a crop, by adding together the unit requirements for 20 acres and 15 acres, the requirements for 35 acres would be obtained. By studying the requirements for the rotation just given, it will be seen that the man unit requirements do not approach either 1, 2 or 3 man capacity very nearly in one month. In March and April, for instance, just three-fourths of the man's time will be taken, so by increasing the acreage one-fourth, or adding 5 acres to each field, a much more satisfactory labor situation is obtained with these crops. Such a change will give results as follows:

Month	Man Units	Horse Units
March	.9425	2.6975
April	.8820	2.8550
May	1.750	3.5575
June	2.4225	3.8050
July	2.8775	3.8450
August	1.6325	2.3450
September	2.5150	3.90
October	1.6150	3.8775
November	1.5124	2.4150
December	1.6025	2.7375
January	.8500	1.0150
February	.6450	.2000

This last arrangement is much better. It will be recalled from a previous diagram (Figure 1) that the best arrangement is to keep every workman regularly employed while he is on the farm.

From the above figures, it will be noticed that one man's time

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is taken for the entire year, another man's time from May 1st to January 1st, and the third man is used about half time in June, July and September. For horse labor, we see 4 horses could nicely handle this rotation.

This fairly represents what will be found on many farms. It is a common rotation in this section, but the acreage in this illustration is probably better worked out than will be usually found. This is probably as convenient an arrangement as could be made with these crops on such a scale. Thus a man is able to know about **when** he needs his extra help and **how much** he needs. Many small changes can be made to add efficiency.

Clover and corn conflict very much, so if a leguminous crop can be substituted for clover to furnish a good feed, the latter may be successfully omitted from the rotation. This has been accomplished in many instances by following wheat with cowpeas. Or, as one farmer successfully handled the matter, by sowing cowpeas broadcast in corn at last cultivation; cutting the corn off in August for silage, and later cutting the cowpeas for hay. Also oats may be cut for hay in June if the July labor schedule is excessively heavy.

The many methods of harvesting corn allows a man much latitude in caring for that crop in the fall. Cowpeas and soybeans, as long as it is thought necessary to cultivate them, apparently will not fit in with corn because of the conflict in the labor schedule in planting and tending these different crops. Cowpeas sown broadcast for hay will work in much better. Thus it is seen that while actual conditions and natural limits on one farm would require that a certain crop be worked a certain way, shifts may be made in rotations which will prove beneficial to the labor schedule. But at best it will not be possible to entirely balance labor by changing the cropping system. Where regular labor is desirable, it will be found necessary to turn to the other production labor for relief.

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TABLE V.—MAN LABOR REQUIREMENTS OF COMMO

MAN HOURS PER ACRE

	Corn	Oats	Wheat	Clover
March	.15	2.92		
April	1.84	1.32		.46
May	6.38	.26		.45
June	6.72	1.11	3.2	3.56
July	2.14	3.50	6.4	.43
August	1.84	1.16	1.62	1.22
September	5.03	.17	2.70	.68
October	.78		4.2	.86
November	3.14		.26	
December	2.55			
January	.55			
February	.48			
Totals	31.60	10.44	18.38	7.66

	Rye	Timothy	Rape	Cowpeas Cult.
April			2.3	2.1
May				
June	2.03	.48		3.3
July	2.00	6.02		4.1
August	6.56			1.9
Sept.	1.96			
Oct.	.70			13.4
Nov.				
Dec.				
Jan.				
Feb.				
Totals	13.25	6.50	2.3	24.8

Showing the average man hours per acre given each mon common farm crops on four farms.

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TABLE VI.—HORSE LABOR REQUIREMENTS OF CROPS.

HORSE HOURS PER ACRE.

	Corn	Oats	Wheat	Clover
March		6.65		
April	4.57	2.93		1.15
May	14.09	.79		.78
June	10.91	.87	3.2	3.38
July	3.25	3.69	6.5	.68
August	1.17	1.28	3.1	.69
September	1.73	.21	5.1	.71
October	.72		7.3	1.04
November	1.77		.5	
December	1.61			
January	.28			
February	.10			
Totals	40.20	16.42	25.7	8.43

	Rye	Timothy	Rape	Cowpeas Cult.	Cowpeas Not Cult.
April			6.4	5.9	
May					3.6
June	2.27	.63		10.7	12.0
July	2.65	9.02		5.1	
August	14.94				
Sept.	3.46				1.2
Oct.	1.27			12.6	5.1
Nov.					2.9
Dec.					.26
Jan.					
Feb.					
Totals	24.59	9.65	6.4	34.3	25.06

Showing for horse labor what Table V shows for man labor.

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TABLE VII.—MAN UNIT REQUIREMENTS OF COMMON CROPS.

Giving the proportion of one man's available time required by acreages from 1 to 20 of common farm crops. Based on previously given figures.. (Tables IV and V).

	CORN												TIMOTHY	
Acres	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	June	July
1	.0018	.0195	.0630	.0447	.0198	.0206	.0590	.0086	.0561	.0641	.0340	.0258	.0032	.0555
2	.0036	.0390	.1260	.0894	.0396	.0412	.1180	.0172	.1122	.1282	.0680	.0516	.0064	.1110
3	.0054	.0585	.1890	.1341	.0594	.0618	.1770	.0258	.1683	.1923	.1020	.0774	.0096	.1665
4	.0072	.0780	.2520	.1788	.0792	.0824	.2360	.0344	.2244	.2564	.1360	.1032	.0128	.2220
5	.0090	.0975	.3150	.2235	.0990	.1030	.2950	.0430	.2805	.3205	.1700	.1290	.0160	.2775
6	.0108	.1170	.3780	.2682	.1188	.1236	.3540	.0516	.3366	.3846	.2040	.1548	.0192	.3330
7	.0126	.1365	.4410	.3129	.1386	.1442	.4130	.0602	.3927	.4487	.2380	.1806	.0224	.3885
8	.0144	.1560	.5040	.3576	.1584	.1648	.4720	.0688	.4488	.5128	.2720	.2064	.0256	.4440
9	.0162	.1755	.5670	.4023	.1782	.1854	.5310	.0774	.5049	.5769	.3060	.2322	.0288	.4995
10	.0180	.1950	.6300	.4470	.1980	.2060	.5900	.0860	.5610	.6410	.3400	.2580	.0320	.5550
11	.0198	.2145	.6930	.4917	.2178	.2266	.6490	.0946	.6171	.7051	.3740	.2838	.0352	.6105
12	.0216	.2340	.7560	.5364	.2376	.2472	.7080	.1032	.6732	.7692	.4080	.3096	.0384	.6660
13	.0234	.2535	.8190	.5811	.2574	.2678	.7670	.1118	.7293	.8333	.4420	.3354	.0416	.7215
14	.0252	.2730	.8820	.6258	.2772	.2884	.8260	.1204	.7854	.8974	.4760	.3612	.0448	.7770
15	.0270	.2925	.9450	.6705	.2970	.3090	.8850	.1290	.8415	.9615	.5100	.3870	.0480	.8325
16	.0288	.3120	1.0080	.7152	.3168	.3296	.9440	.1376	.8976	1.0256	.5440	.4128	.0512	.8880
17	.0306	.3315	1.0710	.7599	.3366	.3502	1.0030	.1462	.9537	1.0897	.5780	.4386	.0544	.9435
18	.0324	.3510	1.1340	.8046	.3564	.3708	1.0620	.1548	1.0098	1.1538	.6120	.4644	.0576	.9990
19	.0342	.3705	1.1970	.8493	.3762	.3914	1.1210	.1634	1.0659	1.2179	.6460	.4902	.0608	1.0545
20	.0360	.3900	1.2600	.8940	.3960	.4120	1.1800	.1720	1.1220	1.2820	.6800	.5160	.0640	1.1100

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TABLE VII (Continued)—**Man Unit Requirements of Common Crops.**

	OATS							WHEAT						RAPE (Broad casted)
Acres	Mar.	Apr.	May	June	July	Aug.	Sept.	June	July	Aug.	Sept.	Oct.	Nov.	Apr.
1	.0359	.0140	.0026	.0074	.0323	.0130	.0020	.0212	.0591	.0181	.0317	.0465	.0046	.0245
2	.0718	.0280	.0052	.0148	.0646	.0260	.0040	.0424	.1182	.0362	.0634	.0930	.0092	.0490
3	.1077	.0420	.0078	.0222	.0969	.0390	.0060	.0636	.1773	.0543	.0951	.1395	.0138	.0735
4	.1436	.0560	.0104	.0296	.1292	.0520	.0080	.0848	.2364	.0724	.1268	.1860	.0184	.0980
5	.1795	.0700	.0130	.0370	.1615	.0650	.0100	.1060	.2955	.0905	.1585	.2325	.0230	.1225
6	.2154	.0840	.0156	.0444	.1938	.0780	.0120	.1272	.3546	.1086	.1902	.2790	.0276	.1470
7	.2513	.0980	.0182	.0518	.2261	.0910	.0140	.1484	.4137	.1267	.2219	.3255	.0322	.1715
8	.2872	.1120	.0208	.0592	.2584	.1040	.0160	.1696	.4728	.1448	.2536	.3720	.0368	.1960
9	.3231	.1260	.0234	.0666	.2907	.1170	.0180	.1908	.5319	.1629	.2853	.4185	.0414	.2205
10	.3590	.1400	.0260	.0740	.3230	.1300	.0200	.2120	.5910	.1810	.3170	.4650	.0460	.2450
11	.3949	.1540	.0286	.0814	.3553	.1430	.0220	.2332	.6501	.1991	.3487	.5115	.0506	.2695
12	.4308	.1680	.0312	.0888	.3876	.1560	.0240	.2544	.7092	.2172	.3804	.5580	.0552	.2940
13	.4667	.1820	.0338	.0962	.4199	.1690	.0260	.2756	.7683	.2353	.4121	.6045	.0598	.3185
14	.5026	.1960	.0364	.1036	.4522	.1820	.0280	.2968	.8274	.2534	.4438	.6510	.0644	.3430
15	.5385	.2100	.0390	.1110	.4845	.1950	.0300	.3180	.8865	.2715	.4755	.6975	.0690	.3675
16	.5744	.2240	.0416	.1184	.5168	.2080	.0320	.3392	.9456	.2896	.5072	.7440	.0736	.3920
17	.6103	.2380	.0442	.1258	.5491	.2210	.0340	.3604	1.0047	.3077	.5389	.7905	.0782	.4165
18	.6462	.2520	.0468	.1332	.5814	.2340	.0360	.3816	1.0638	.3258	.5706	.8370	.0828	.4410
19	.6821	.2660	.0494	.1406	.6137	.2470	.0380	.4028	1.1229	.3439	.6023	.8835	.0874	.4655
20	.7180	.2800	.0520	.1480	.6460	.2600	.0400	.4240	1.1820	.3620	.6340	.9300	.0920	.4900

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TABLE VII (Continued)—**Man Unit Requirements of Common Crops.**

	CLOVER							RYE				
Acres	Apr	May	June	July	Aug.	Sept.	Oct.	June	July	Aug.	Sept.	Oct.
1	.0049	.0044	.0236	.0039	.0136	.0079	.0095	.0135	.0185	.0735	.0230	.0076
2	.0098	.0088	.0472	.0078	.0272	.0158	.0190	.0270	.0370	.1470	.0460	.0152
3	.0147	.0132	.0708	.0117	.0408	.0237	.0285	.0405	.0555	.2205	.0690	.0228
4	.0196	.0176	.0944	.0156	.0544	.0316	.0380	.0540	.0740	.2940	.0920	.0304
5	.0245	.0220	.1180	.0195	.0680	.0395	.0475	.0675	.0925	.3675	.1150	.0380
6	.0294	.0264	.1416	.0234	.0816	.0474	.0570	.0810	.1110	.4410	.1380	.0456
7	.0343	.0308	.1652	.0273	.0952	.0553	.0665	.0945	.1295	.5145	.1610	.0532
8	.0392	.0352	.1888	.0312	.1088	.0632	.0760	.1080	.1480	.5880	.1840	.0608
9	.0441	.0396	.2124	.0351	.1224	.0711	.0855	.1215	.1665	.6615	.2070	.0684
10	.0490	.0440	.2360	.0390	.1360	.0790	.0950	.1350	.1850	.7350	.2300	.0760
11	.0539	.0484	.2596	.0429	.1496	.0869	.1045	.1485	.2035	.8085	.2530	.0836
12	.0588	.0528	.2832	.0468	.1632	.0948	.1140	.1620	.2220	.8820	.2760	.0912
13	.0637	.0572	.3068	.0507	.1768	.1027	.1235	.1755	.2405	.9555	.2990	.0988
14	.0686	.0616	.3304	.0546	.1904	.1106	.1330	.1890	.2590	1.0290	.3220	.1064
15	.0735	.0660	.3540	.0585	.2040	.1185	.1425	.2025	.2775	1.1025	.3450	.1140
16	.0784	.0704	.3776	.0624	.2176	.1264	.1520	.2160	.2960	1.1760	.3680	.1216
17	.0833	.0748	.4012	.0663	.2312	.1343	.1615	.2295	.3145	1.2495	.3910	.1292
18	.0882	.0792	.4248	.0702	.2448	.1422	.1710	.2430	.3330	1.3230	.4140	.1368
19	.0931	.0836	.4484	.0741	.2584	.1501	.1805	.2565	.3515	1.3965	.4370	.1444
20	.0980	.0880	.4720	.0780	.2720	.1580	.1900	.2700	.3700	1.4700	.4680	.1520

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TABLE VII (Continued)—**Man Unit Requirements of Common Crops.**

	COWPEAS (Cultivated)					COWPEAS (Not Cultivated)					
Acres	Apr	June	July	Aug.	Oct.	May	June	Sept.	Oct.	Nov.	Dec.
1	.0223	.0220	.0378	.0215	.1485	.0167	.0440	.0152	.0670	.1125	.0196
2	.0446	.0440	.0756	.0430	.2970	.0334	.0880	.0304	.1340	.2250	.0392
3	.0669	.0660	.1134	.0645	.4455	.0501	.1320	.0456	.2010	.3375	.0588
4	.0892	.0880	.1512	.0860	.5940	.0668	.1760	.0608	.2680	.4500	.0784
5	.1115	.1100	.1890	.1075	.7425	.0835	.2200	.0760	.3350	.5625	.0980
6	.1338	.1320	.2268	.1290	.8910	.1002	.2640	.0912	.4020	.6750	.1176
7	.1561	.1540	.2646	.1505	1.0395	.1169	.3080	.1064	.4690	.7875	.1372
8	.1784	.1760	.3024	.1720	1.1880	.1336	.3520	.1216	.5360	.9000	.1568
9	.2007	.1980	.3402	.1935	1.3365	.1503	.3960	.1368	.6030	1.0125	.1764
10	.2230	.2200	.3780	.2150	1.4850	.1670	.4400	.1520	.6700	1.1250	.1960
11	.2453	.2420	.4158	.2365	1.6335	.1837	.4840	.1672	.7370	1.2375	.2156
12	.2676	.2640	.4536	.2580	1.7820	.2004	.5280	.1824	.8040	1.3500	.2352
13	.2899	.2860	.4914	.2795	1.9305	.2171	.5720	.1976	.8710	1.4625	.2548
14	.3122	.3080	.5292	.3010	2.0790	.2338	.6160	.2128	.9380	1.5750	.2744
15	.3345	.3300	.5670	.3225	2.2275	.2505	.6600	.2280	1.0050	1.6875	.2940
16	.3568	.3520	.6048	.3440	2.3760	.2672	.7040	.2432	1.0720	1.8000	.3136
17	.3791	.3740	.6426	.3655	2.5245	.2839	.7480	.2584	1.1390	1.9125	.3332
18	.4014	.3960	.6804	.3870	2.6730	.3006	.7920	.2736	1.2060	2.0250	.3528
19	.4237	.4180	.7182	.4085	2.8215	.3173	.8360	.2888	1.2730	2.1375	.3724
20	.4460	.4400	.7560	.4300	2.9700	.3340	.8800	.3040	1.3400	2.2500	.3920

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TABLE VIII.—HORSE UNIT REQUIREMENTS OF COMMON CROPS.

Giving the proportion of one horse's available time required by acres from 1 to 20 of common farm crops, based on average time given to crops and labor requirements of those crops (Tables IV and VI).

	CORN											COWPEAS (Cultivated)			
Acres	Apr	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Apr.	June	July	Oct.
1	.0603	.1280	.0890	.0354	.0176	.0280	.0123	.0753	.1095	.0406	.0080	.0778	.0873	.0556	.2160
2	.1206	.2560	.1780	.0708	.0352	.0560	.0246	.1506	.2190	.0812	.0160	.1556	.1746	.1112	.4320
3	.1809	.3840	.2670	.1062	.0528	.0840	.0369	.2259	.3285	.1218	.0240	.2334	.2619	.1668	.6480
4	.2412	.5120	.3560	.1416	.0704	.1120	.0492	.3012	.4380	.1624	.0320	.3112	.3492	.2224	.8640
5	.3015	.6400	.4450	.1770	.0880	.1400	.0615	.3765	.5475	.2030	.0400	.3890	.4365	.2780	1.0800
6	.3618	.7680	.5340	.2124	.1056	.1680	.0738	.4518	.6570	.2436	.0480	.4668	.5238	.3336	1.2960
7	.4221	.8960	.6230	.2478	.1232	.1960	.0861	.5271	.7665	.2842	.0560	.5446	.6111	.3892	1.5120
8	.4824	1.0240	.7120	.2832	.1408	.2240	.0984	.6024	.8760	.3248	.0640	.6224	.6984	.4448	1.7280
9	.5427	1.1520	.8010	.3186	.1584	.2520	.1107	.6777	.9855	.3654	.0720	.7002	.7857	.5004	1.9440
10	.6030	1.2800	.8900	.3540	.1760	.2800	.1230	.7530	1.0950	.4060	.0800	.7780	.8730	.5560	2.1600
11	.6633	1.4080	.9790	.3894	.1936	.3080	.1353	.8283	1.2045	.4466	.0880	.8558	.9603	.6116	2.3760
12	.7236	1.5360	1.0680	.4248	.2112	.3360	.1476	.9036	1.3140	.4872	.0960	.9336	1.0476	.6672	2.5920
13	.7839	1.6640	1.1570	.4602	.2288	.3640	.1599	.9789	1.4235	.5278	.1040	1.0114	1.1349	.7228	2.8080
14	.8442	1.7920	1.2460	.4956	.2464	.3920	.1722	1.0542	1.5330	.5684	.1120	1.0892	1.2222	.7784	3.0240
15	.9045	1.9200	1.3350	.5310	.2640	.4200	.1845	1.1295	1.6425	.6090	.1200	1.1670	1.3095	.8340	3.2400
16	.9648	2.0480	1.4240	.5664	.2816	.4480	.1968	1.2048	1.7520	.6496	.1280	1.2448	1.3968	.8896	3.4560
17	1.0251	2.1760	1.5130	.6018	.2992	.4760	.2091	1.2801	1.8615	.6902	.1360	1.3226	1.4841	.9452	3.6720

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TABLE VIII (Continued)— **Horse Unit Requirements of Common Crops.**

	OATS							WHEAT						RAPE (Broadcast)
Acres	Mar.	Apr.	May	June	July	Aug.	Sept.	June	July	Aug.	Sept.	Oct.	Nov.	Apr.
1	.1079	.0387	.0072	.0071	.0402	.0192	.0034	.0285	.0708	.0466	.0825	.1250	.0213	.0844
2	.2158	.0774	.0144	.0142	.0804	.0384	.0068	.0570	.1416	.0932	.1650	.2500	.0426	.1688
3	.3237	.1161	.0216	.0213	.1206	.0576	.0102	.0855	.2124	.1398	.2475	.3750	.0639	.2532
4	.4316	.1548	.0288	.0284	.1608	.0768	.0136	.1140	.2832	.1864	.3300	.5000	.0852	.3376
5	.5395	.1935	.0360	.0355	.2010	.0960	.0170	.1425	.3540	.2330	.4125	.6250	.1065	.4220
6	.6474	.2322	.0432	.0426	.2412	.1152	.0204	.1710	.4248	.2796	.4950	.7500	.1278	.5064
7	.7553	.2709	.0504	.0497	.2814	.1344	.0238	.1995	.4956	.3262	.5775	.8750	.1491	.5908
8	.8632	.3096	.0576	.0568	.3216	.1536	.0272	.2280	.5664	.3728	.6600	1.0000	.1704	.6752
9	.9711	.3483	.0648	.0639	.3618	.1728	.0306	.2565	.6372	.4194	.7425	1.1250	.1917	.7596
10	1.0790	.3870	.0720	.0710	.4020	.1920	.0340	.2850	.7080	.4660	.8250	1.2500	.2130	.8440
11	1.1869	.4257	.0792	.0781	.4422	.2112	.0374	.3135	.7788	.5126	.9075	1.3750	.2343	.9284
12	1.2948	.4644	.0864	.0852	.4824	.2304	.0408	.3420	.8496	.5592	.9900	1.5000	.2556	1.0128
13	1.4027	.0531	.0936	.0923	.5226	.2496	.0442	.3705	.9204	.6058	1.0725	1.6250	.2769	1.0972
14	1.5106	.5418	.1008	.0994	.5628	.2688	.0476	.3990	.9912	.6524	1.1550	1.7500	.2982	1.1816
15	1.6185	.5805	.1080	.1065	.6030	.2880	.0510	.4275	1.0620	.6990	1.2375	1.8750	.3195	1.2660
16	1.7264	.6192	.1152	.1136	.6432	.3072	.0544	.4560	1.1328	.7456	1.3200	2.0000	.3408	1.3504
17	1.8343	.6579	.1224	.1207	.6834	.3264	.0578	.4845	1.2036	.7922	1.4025	2.1250	.3621	1.4348
18	1.9422	.6966	.1296	.1278	.7236	.3456	.0612	.5130	1.2744	.8388	1.4850	2.2500	.3834	1.5192
19	2.0501	.7353	.1368	.1349	.7638	.3648	.0646	.5415	1.3452	.8854	1.5675	2.3750	.4047	1.6036
20	2.1580	.7740	.1440	.1420	.8040	.3840	.0680	.5700	1.4160	.9320	1.6500	2.5000	.4260	1.6880

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TABLE VIII (Continued)— **Horse Unit Requirements of Common Crops.**

	CLOVER							RYE					TIMOTHY	
Acres	Apr.	May	June	July	Aug.	Sept.	Oct.	June	July	Aug.	Sept.	Oct.	June	July
1	.0152	.0071	.0276	.0074	.0104	.0115	.0178	.0185	.0289	.2147	.0560	.0218	.0051	.0984
2	.0304	.0142	.0552	.0148	.0208	.0230	.0356	.0370	.0578	.4294	.1120	.0436	.0102	.1968
3	.0456	.0213	.0828	.0222	.0312	.0345	.0534	.0555	.0867	.6441	.1680	.0654	.0153	.2952
4	.0608	.0284	.1104	.0296	.0416	.0460	.0712	.0740	.1156	.8588	.2240	.0872	.0204	.3936
5	.0760	.0355	.1380	.0370	.0520	.0575	.0890	.0925	.1445	1.0735	.2800	.1090	.0255	.4920
6	.0912	.0426	.1656	.0444	.0624	.0690	.1068	.1110	.1734	1.2882	.3360	.1308	.0306	.5904
7	.1064	.0497	.1932	.0518	.0728	.0805	.1246	.1295	.2023	1.5029	.3920	.1526	.0357	.6888
8	.1216	.0568	.2208	.0592	.0832	.0920	.1424	.1480	.2312	1.7176	.4480	.1744	.0408	.7872
9	.1368	.0639	.2484	.0666	.0936	.1035	.1602	.1665	.2601	1.9323	.5040	.1962	.0459	.8856
10	.1520	.0710	.2760	.0740	.1040	.1150	.1780	.1850	.2890	2.1470	.5600	.2180	.0510	.9840
11	.1672	.0781	.3036	.0814	.1144	.1265	.1958	.2035	.3179	2.3617	.6160	.2398	.0561	1.0824
12	.1824	.0852	.3312	.0888	.1248	.1380	.2136	.2220	.3468	2.5764	.6720	.2616	.0612	1.1808
13	.1976	.0923	.3588	.0962	.1352	.1495	.2314	.2405	.3757	2.7911	.7280	.2834	.0663	1.2792
14	.2128	.0994	.3864	.1036	.1456	.1610	.2492	.2590	.4046	3.0058	.7840	.3052	.0714	1.3776
15	.2280	.1065	.4140	.1110	.1560	.1725	.2670	.2775	.4335	3.2205	.8400	.3270	.0765	1.4760
16	.2432	.1136	.4416	.1184	.1664	.1840	.2848	.2960	.4624	3.4352	.8960	.3488	.0816	1.5744
17	.2584	.1207	.4692	.1258	.1768	.1955	.3026	.3145	.4913	3.6499	.9520	.3706	.0867	1.6728
18	.2736	.1278	.4968	.1332	.1872	.2070	.3204	.3330	.5202	3.8646	1.0080	.3924	.0918	1.7712
19	.2888	.1349	.5244	.1406	.1976	.2185	.3382	.3515	.5491	4.0793	1.0640	.4142	.0969	1.8696
20	.3040	.1420	.5520.	1480	.2080.	.2300	.3560	.3700	.5780	4.2940	1.1200	.4360	.1020	1.9680

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TABLE VIII (Continued)
Horse Unit Requirements of Common Crops.

Acres	COWPEAS (not cult.)					
	May	June	Sept.	Oct.	Nov.	Dec.
1	.0327	.0978	.0194	.0873	.1234	.0177
2	.0654	.1956	.0388	.1746	.2468	.0354
3	.0981	.2934	.0582	.2619	.3702	.0531
4	.1308	.3912	.0776	.3492	.4936	.0708
5	.1635	.4890	.0970	.4365	.6170	.0885
6	.1962	.5868	.1164	.5238	.7404	.1062
7	.2289	.6846	.1358	.6111	.8638	.1239
8	.2616	.7824	.1552	.6984	.9872	.1416
9	.2943	.8802	.1746	.7857	1.1106	.1593
10	.3270	.9780	.1940	.8730	1.2340	.1770
11	.3597	1.0758	.2134	.9603	1.3574	.1947
12	.3924	1.1736	.2328	1.0476	1.4808	.2124
13	.4251	1.2714	.2522	1.1349	1.6042	.2301
14	.4578	1.3692	.2716	1.2222	1.7276	.2478
15	.4905	1.4670	.2910	1.3095	1.8510	.2655
16	.5232	1.5648	.3104	1.3968	1.9744	.2832
17	.5559	1.6626	.3298	1.4841	2.0978	.3009
18	.5886	1.7604	.3492	1.5714	2.2212	.3186
19	.6213	1.8582	.3686	1.6587	2.3446	.3363
20	.6540	1.9560	.3880	1.7460	2.4680	.3540

OTHER PRODUCTION LABOR.

On the farms under discussion the per cent of time given to the class of work called "other production labor" was fairly uniform with man labor, but with horses it was more irregular. (Table 3). Approximately 55% of a man's time is given to other production labor. On a strictly grain farm this class of labor is probably not so prominent; but on the diversified farm, if only to the slightest degree diversified, this class of labor plays a more or less important part.

A study of some conditions on these farms will well illustrate the importance of this class of labor. Approximately 3.3 per cent of the total labor on Farms 1 and 2 was given to cows. In this case just enough cows were kept to supply home needs. Farm 1 kept two cows at less than half the labor cost per cow that Farm 2 incurred with one cow, thus showing that a very large difference may exist in simple operations on different farms. It is these little things which help to mould final results.

When we look at Farms 3 and 4, we find 20% of the total labor devoted to cows. This shows the importance of the dairy phase of the work on these farms. This was also found to directly affect the amount of labor which it is possible to give to the work stock

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each day. The delivering of milk each day played no small part in increasing the average hours per day worked by the horses. An interesting comparison may be made in the case of labor cost per cow on Farms 3 and 4 (Table 2).

The extra labor cost per cow on Farm 3 was due entirely to the difference in convenience of handling the dairy work on the two farms. Because of inadequate arrangements on Farm 3, the milking was done one-fourth mile from the house. Farm 4 was fairly well arranged for dairying. It should easily be possible to reduce the labor cost on this farm about 20%. But the extra cost of 3 cents per cow per day on Farm 3, is quite a price to pay for inconvenience, to say nothing of the profit that might have been made on this wasted labor, if it could have been used at some other phase of farm work. The excess cost would mean in a year's time, slightly over $10.00 per head, an item not to be overlooked in a business like dairying, where a man is simply changing one kind of product into another kind, and his profits are determined by the economy with which he makes the change. The margins in dairying are usually much narrower than in the growing of crops, so that $10.00 per year might make quite a good deal of difference in the profit per cow. Also $10.00 per cow would be pretty good interest on a modern dairy barn and fencing which would allow the milking to be done nearer where the milk is to be handled. Thus it will be seen that small things in the other productive labor class can, by being allowed to take their own course, lessen materially the efficiency of labor; and when the importance of the labor done on the farm outside the field is considered (Table 3) we can easily see how efficient handling of this portion of farm labor may change the results of a year's work very materially.

The breeding and feeding of other classes of live stock are important factors in the other production labor class. The production of fall pigs, winter lambs, attention given to poultry—such things will help very materially in furnishing regular work when field work is not obtainable. The hauling of manure and ferilizers in the winter months will give employment to both man and horse. Special attention to the cleaning and grading of seeds, the killing, curing and marketing of meat, more attention to the orchard and garden products in a way that will make them more salable, all such efforts will aid very materially in the successful filling in of the periods of less demand for labor. It will usually be found more difficult to handle horse labor satisfactorily.

At this point attention is called to the importance of brood

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mares as work animals. The producing of both spring and fall colts will add no small item to the returns from work stock for the year. Where an abundance of warm barn space and good feed can be supplied, greater benefit in this respect will usually be received by producing fall colts. This is because the mare will not be so handicapped for work in the busy spring season as she is with a spring foal. Also she has little to do in fall and winter, so the care of a foal would keep her earning her keep. Of course fall colts would be no object to the man who has a surplus of work stock in spring, but for those who expect to keep just enough to do the work under the most difficult arrangements, such a means of using work stock in otherwise idle seasons would be a great help. Great care should be taken to select a good grade of mares as well as sure breeders. The greater cost of keeping breeding mares coupled with a somewhat less amount of work done makes readily salable products as well as regular production almost imperative. A brood mare as a work horse will usually pay for her keep in labor so that the profit realized on the foal is clear gain. In addition to this means of utilizing horse labor, another field is opening up which bids fair to aid materially a certain group of farmers.

At the present rate of development the farm tractor will probably soon be able to take the place of part of the work stock on farms of 200 acres or more, thus aiding materially in handling the labor schedule with regard to horses.

MAINTENANCE.

The maintenance factor, as has been mentioned, will under normal conditions be fairly stable. This will be a little better understood perhaps, when we mention the fact that it will take during the year about so much time to care for a definite amount of farm equipment, or of buildings and fences. As there is then a certain amount of this work to do, the best results will be obtained by arranging for all of this work that can be shifted at all, to be done at times when other work is not crowding. The work stock is one phase of this class of work that will come regularly. On the four farms this class of labor required approximately 5 per cent of the total labor of the farm (Table 3). Work of this nature must be done daily, but the repairing of machinery, buildings and fences can, to a certain extent, be shifted—if a man will plan his work ahead. This shifting will add its small part to the balancing of labor.

The different things in the foregoing study which reveal weak

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places in common labor practices brings out the strong need of more study by the farmer of his individual condition in an effort to make labor a more efficient factor in his farm management. Every man should ask himself questions like the following with regard to his operations:

Is it possible for me to change my cropping system to better distribute my labor and cause fewer conflicts? Can I find some opportunity here for introducing more other production labor for men and horses out of crop season? Is my up-keep labor planned so that it is a minimum in the busy crop season?

One of the greatest aids in the bettering of the labor situation is to plan the work ahead. This planning ahead may be called making a "labor schedule." If a man can prepare before-hand a carefully made plan of the season's work, he will often be able to greatly relieve the congestion of certain seasons, and also keep his men employed at profitable labor, in what would otherwise be dull periods. The most successful farmers are those who figure ahead, and the more experience they have in this and the more detailed their plans the better they succeed. With a well planned labor schedule the manager will never send the men to cut brush along the fence rows when the binder must be overhauled for wheat cutting tomorrow, or the day after, or the granaries made ready for threshing.

When planting time begins the first day or two of good weather will not be wasted in getting seed cleaned, or getting the machinery in running order. Such a man is able to take advantage of every moment and he is the one who shows results in the end.

The problem of labor distribution or the proper adjustment of labor equipment and labor requirements is thus seen to directiy affect the profits of farm operations, and the degree to which these two factors are adjusted will directly affect, and in no small part determine, the amount of a manager's success.

SUMMARY.

1. Attention is again called to the fact that the presentation of this material is made not because it is an exhaustive treatment of methods of solving certain problems, but rather because it gives strong evidence from the limited sources at hand, of what some of these problems are. The object then is to present the problems as they have shaped themselves in the investigation up to this time and to suggest lines along which it will be possible to work in solving these problems.

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2. The length of day worked varied in the case of man labor from 7.8 hours in February to 11.7 hours in June, and in the case of horse labor from 1.2 hours in January to 6.4 hours in May. The average was as follows: man 9.9 hours and horse 3.9 hours.

3. The labor required for care of work stock varied from 4½ to 7% of the total labor used on the farm. The cost of caring for horses, allowing 15 cents per hour for man and 10 cents per hour for horse labor, was from 4c to 6c per day per horse.

4. The three classes of labor on the farms studied were divided up fairly uniformly as follows: Maintenance 17%, Crop 28%, Other Production 55%. This uniformity was with man labor only. With horse labor the variation was marked. Maintenance varied from 6 to 21%, Crop 51 to 69%, and Other Production from 19 to 41%. This means that one-sixth of the work put in on the farm in these cases was given to Maintenance. In other words, no profit was realized on this portion of the labor. Effort should be made to reduce the per cent of labor given to Maintenance by increasing Other Production Labor. This would increase profits and at the same time would not decrease the amount of labor given to up-keep of the business.

5. The average workman on the farms studied worked 3272.3 hours per year, 931 hours of this time were given to Crop Production.

6. With reference to horse labor 1216.6 hours per horse were worked during the year, while 705.9 hours of this time were given to Crop Production. This illustrates the dependence of horses on crop work and the independence of man labor on this same class. The importance of making arrangements for other means of using horses than on field crops is evident. Also the arrangement of conveniences for saving time in caring for stock, etc., by the men. When 72% of a man's time is spent out of the field, the arrangement of buildings, lots, etc., may affect profits to a large extent.

7. Assuming that the crops received attention whenever they required it, the following figures give the man and horse labor requirements per acre of some common crops:

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	Man Hours	Horse Hours
Corn	31.60	40.20
Oats	10.44	16.42
Wheat	18.38	25.70
Clover	7.66	8.43
Rye	13.25	24.59
Timothy	6.50	9.65
Rape	2.30	6.40
Cowpeas (cultivated)	24.80	34.30
Cowpeas (uncultivated)	22.78	25.06

8. A study of the labor requirements of crops by months gives the basis for fitting crops together from the standpoint of labor distribution.

9. A comparison of the labor requirements of crops and the number of hours available for crop work each month per man or per horse will show the men and horses required for a certain crop or the acreage of a certain rotation that a definite number of men and horses can handle.

10. Several problems are thus open for consideration. (a) An arrangement of crop rotations to give a minimum of conflicting operations is imperative. (b) More Other Production labor is essential in giving regular employment to workmen. (c) Efforts must be made to supply regular employment other than Crop Labor for farm work stock.

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(1) In the service of the U. S. Department of Agriculture.

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NUTRIENTS REQUIRED FOR MILK PRODUCTION.

C. H. ECKLES.

According to the latest census the dairy cows in the United States number nearly twenty millions. The value of the feed required each year by these animals represents an enormous sum. It is recognized that proper methods of feeding are necessary in order that this large amount of feed be used to the best advantage. This problem of economical feeding has received the attention of numerous investigators for the past fifty years.

The first attempt to assign to different feeding stuffs their true value for feeding purposes is shown in the so-called hay equivalents or hay values which were quite commonly advocated in Europe prior to the year 1860. Good meadow hay was taken as the unit and all other feeds were given relative values. These values were based upon the results of practical experiments. This method of judging the nutritive values of feeds did not prove entirely satisfactory, probably because of the limited amount of experimental work upon which it was based and because the unit for comparison, meadow hay, was too variable. A modification of this system is being used at present in some of the north European countries. Each foodstuff is given a value of a certain number of units, which represents approximately the relative nutritive values as determined by the carefully conducted feeding experiments of Fjord, a Danish investigator. These "food units" are convenient for use when buying food or when estimating the economy of production for herds or individual cows, dealing as they do entirely with the nutritive values, not the market values. Grouven[1] in 1859 is said to be the first man to suggest compounding rations with reference to the actual amounts of the different nutrients they contain—crude protein, carbohydrates, and ether extract.

Henneberg and Stohmann at Weende later showed that these constituents were not digested in the same proportions for all feeds, so it was suggested that only the digestible nutrients be used in the calculation of rations. As a result of Henneberg and Stohmann's work, Wolff, in 1864 published the first feeding standard, based upon the amount of digestible nutrients contained in feeding stuffs.

1. Exp. Station Record, v. 4, Translation from Prof. J. Kuhn.

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This standard was deduced from the results of a large number of experiments at different times by different observers. It calls for 24.0 lbs. dry matter, and digestible nutrients to the extent of 2.5 lbs. protein, 12.50 lbs. carbohydrates, and .4 lbs. ether extract for a cow weighing 1000 pounds. While this seems to meet the requirements of a good average dairy cow fairly well, it is criticised for not making any allowance for a very heavy producer or a light producer.

Prof. Julius Kuhn of the Halle Experiment Station,[1] in 1861 was the first scientist of prominence to question the advisability of feeding all cows the same, irrespective of production or of kind or quality of feed. Later he proposed a standard which is somewhat more flexible than Wolff's and has the nutrients arranged a little differently. He objected to the classifying of all nitrogen compounds under the head of protein and considering them as having the same nutritive value. His standard was as follows:

Dry Matter, 20 to 23.5 lbs.
Digestible Albuminoids, 1.5 to 2.4 lbs.
Digestible Carbohydrates and Amides, 12 to 14 lbs.

While Kuhn apparently had good reasons for dividing the protein in this way, his classification did not prove popular.

In 1897, Dr. C. Lehmann of Berlin, modified the Wolff standard to meet the criticism of Kuhn and formulated what is known as the Wolff-Lehmann standard. In this the kinds and amounts of nutrients are varied with the quantity of milk produced.

WOLFF-LEHMANN STANDARD.

	Dry Matter Lbs.	Digestible Nutrients		
		Protein Lbs.	Carbohydrates Lbs.	Ether Extract Lbs.
11 lbs. milk.....	25.0	1.6	10.0	.3
16½ lbs. milk.....	27.0	2.0	11.0	.4
22 lbs. milk.....	29.0	2.5	13.0	.5
27½ lbs. milk.....	32.0	3.3	13.0	.8

This standard is probably an improvement on the original, still as Haecker has pointed out,[2] it is not clear why the nutrients

[1]loc. cit.
[2]Haecker, T. L. Bulletin 79, Minn. Exp. Station.

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should not increase in the same proportion as the milk yield. For example the same amount of carbohydrates is prescribed for a cow giving 27½ pounds of milk as to the one giving 22 pounds of milk.

During the winter of 1894-5 Haecker[1] fed 12 cows a fixed ration for 154 days during which time a full flow of milk and yield of butter-fat is said to have been secured. From this record he attempted to determine whether or not the Wolff-Lehmann standard calls for an excess of nutrients. The gain in weight for this period was about 29 pounds per cow or about .2 pounds per head a day. Four of the twelve were two or three-year-old heifers. All the cows were bred during the experiment, at periods varying from 6 weeks to 4 months before its conclusion, so probably all or nearly all the cows were carrying calves. The cows were fed an average of about 12 pounds concentrates each per day along with all they would take of a mixture of timothy hay, 16 parts, and roots, 10 parts, or timothy hay, 18 parts, and silage, 14 parts. The roughage consumed amounted to about 24 pounds per head daily. The daily yield of milk was 26 pounds, testing 4.1% fat. The average weight of the cows was 956 pounds, and the digestible nutrients consumed daily were 2.00 pounds protein, 12.46 pounds carbohydrates and .67 pounds fat, or calculated on the basis of 1000 pounds live weight:

2.03 lbs. protein; 12.77 lbs. carbohydrates; .565 lbs fat.

The Wolff-Lehmann standard under similar conditions prescribes:

3.08 lbs. protein;.....13.00 lbs. carbohydrates .72 lbs. fat.

It will be seen that Haecker's cows used a smaller amount of these nutrients all the way through than is provided for by the standard, the greatest difference being in the protein. Under ordinary dairy conditions in this country he is apparently justified in his assumption that the Wolff-Lehmann standard prescribes an excess of nutrients. However, if the fact be taken into account that these cows were heavily fed on concentrates and that the digestible nutrients contained therein are worth considerably more than those from roughage as has been found by Kellner and Armsby, the results lose much of their significance. His data also shows that the nutrients required for a pound of milk increase with the richness of the milk. Using this data, Haecker formulated a standard for milk of varying richness. More recently these figures have been somewhat modified in the direction of making the requirements slightly higher for milk low in fat and lower for milk rich in fat.

[1]Haecker, T. L. Bulletins 67, 79, Minn. Exp. Station.

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The work of Kellner[1] and Armsby[2] shows that the plan of using digestible nutrients regardless of their source as a basis for feeding standards is inaccurate. Digestible nutrients in themselves show merely the amounts of the feed or ration which are absorbed through the walls of the alimentary tract. No allowance is made for the energy required for digestion and assimilation. Consequently those feeds which are difficult to digest, ordinarily classed as roughness, when compared with concentrates show a greater efficiency than they really possess. Armsby has found that timothy hay with 57% as much digestible material as corn meal, was worth, for flesh or fat production, only 37 % as much as the corn meal.

The question then arises: how can a feeding standard be formulated so as to represent accurately the true feeding value of the different foodstuffs? Kellner carried out extensive investigations to determine this point. He first took some of the pure nutrients such as protein, carbohydrates and fat and determined the nutritive value of each by feeding trials. These results he expressed in the form of heat value or calories. The assumption throughout is that the heat or energy value of the nutrients minus the energy required to prepare the food for use, that is to build up milk solids or body tissue, represents the true nutritive value, or production value. He found with mature fattening cattle the production values of the pure nutrients per pound to be as follows:

Digestible proteids	1016 Calories
Digestible starch or crude fibre	1071 Calories
Digestible cane sugar	812 Calories
Digestible fat:	
In coarse fodders and roots	2041 Calories
In grains and by-products	2273 Calories
In feeds with over 5% fat	2585 Calories

The next step was to apply the values so obtained to the digestible nutrients of the different feeds and compare the computed value of each feed with the real value as determined by actual feeding trials. It was found possible to estimate with a fair degree of accuracy the production value of the concentrated feeds by means of these factors. However, with those feeds containing a higher proportion of fiber this method was not reliable on account of the fact that a larger amount of energy was required for digestion. It was found that

[1]Kellner, Die Ernahrung der Landwirtschaftliches Nutztiere, Chapter IV.
[2]Armsby, Dr. H. P. Bulletins 71, 84, Pa. Exp. Station.

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the energy expended in digestion was directly proportional to the amount of crude fibre present, so by deducting 617 calories for each pound of crude fibre the computed value was brought very close to the real value. This method consists then of multiplying the digestible nutrients by the caloric value of each nutrient and then making a deduction for the crude fibre.

Armsby[1] has formulated from his own and Kellner's work a system of stating the nutrients of feeding stuffs which takes the facts mentioned into account and at the same time makes it possible to express the results in a simple form. He considers that the amides, which with the albuminoids go to make up the total protein, are not as valuable as the albuminoids for nutritive purposes. He classes nutrients under two heads, those which go to form nitrogenous substances, and second, those used for heat, fat production and energy. The amount of digestible albuminoids is the measure for one and the total heat value of the nutrients used for the other. Armsby then expresses the nutritive value in two terms, digestible protein (albuminoids) and energy value. He introduces the term "therm" as a convenient term to represent 1000 calories.

He tentatively suggests, in the absence of definite experimental data, that the total energy necessary for the production of 1 pound of average milk containing 4 per cent fat and 13 percent solids, be placed at .3 therm of production value of the feed. This is in addition to that required by the animal for maintenance. Included in this ration there should be digestible amide-free protein to the extent of .05 pounds for each pound of milk. He recognizes the fact that there is necessarily a variation in the requirements with the richness of the milk but considers that sufficient data is not available to justify an attempt to formulate a standard for milk of varying richness.

Savage[2] in a recent publication compares the nutrients used by twelve cows in the Cornell University herd for periods of 142 days during two seasons with the requirements as given by Haecker and Armsby. As a result of these investigations he also suggests a modified form of Haecker's feeding standard. This increases the amount of protein by 35% and the general nutriment 10%.

[1] Armsby, Bulletin 71, Pa. Exp. Station.
[2] Savage, E. S., Bulletin 323, Cornell University Exp. Station.

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PLAN OF EXPERIMENTS.

In the course of some investigations a portion of which have already been published[1], data was taken in such a way as to make it possible to study accurately the question of total nutrients required for milk production with five Jersey cows. Further plans were then made for the purpose of securing similar data taken under the same conditions but for animals producing milk with a lower per cent of fat and solids. As a result data is now available covering complete lactation periods of one year each for 8 animals with an average per cent of fat ranging from 3.4 to 6.0. In addition data is available for others taken for shorter intervals.

The taking of the data for each animal began a few days after calving. From this time on all feed received was weighed and analyzed and all the milk produced likewise sampled and subjected to chemical analysis. In this way the data obtained shows the total constituents received in the form of feed and the total production of milk and of each constituent. The ration fed was one generally used in this herd and was the same for all the animals. The amount fed each individual animal was regulated by the live weight. Each cow was given as much feed as she would take so long as it was used for milk production. If she showed a gain in live weight it was taken as an indication that more feed was being given than necessary to support the milk production.

A digestion trial covering 10 days time was made with five of the animals when they were at the maximum milk production. All the cows used in this investigation were kept farrow during the period of the experiment. A maintenance trial covering from 120 to 180 days was made with seven of the animals, in order that it might be determined specifically how much nutrients were required to maintain the particular animals. A second digestion trial was carried on while the animals were on maintenance in order to determine if the coefficient of digestibility changed when the ration is reduced from that necessary for heavy milk production to that used for maintenance. In this way it was possible to determine for each animal the actual maintenance (for 5 of the 8 cows used), the feed required for production, and the total production of milk and of each constituent of the milk.

[1]Research Bulletin No. 2, Missouri Exp. Station.

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The food of the dairy cow may be used in four ways:

1. For growth or gain in weight;
2. For development of a fetus;
3. For maintenance;
4. For milk production.

The data taken in this investigation does not concern itself with Nos. 1 and 2 in the above. It is necessary, however, to either eliminate or measure these two factors. In these investigations they are both eliminated; the first by using only mature cows and regulating the ration so that they neither gained or lost in body weight; the second, by keeping the cows farrow.

Maintenance Requirement. A large amount of work has been done in regard to this factor in animal nutrition and, for cattle, the results by different investigators seem to be so consistent that it is evident that reasonable accuracy has been reached. It is not the purpose to give in detail, the data in regard to maintenance requirements for the animals used in this investigation. A portion has already been published[1] and only a summary will be given of this and the remainder, which as yet is not published. It is sufficient to say that the results on the whole compare closely with the standard as published by Armsby.[2] It was considered advisable in making our experiments to determine the maintenance requirements for each animal individually so far as practical rather than to use average figures. Furthermore, the maintenance trials were conducted with exactly the same ration, including the ratio between the grain and roughage, as used when the animals were fed for milk production. In this way it is possible to estimate the proportion of the ration fed to the cows while in milk that was necessary for maintaining the animal.

Requirements for Milk Production. The object in carrying out the investigation here reported was to obtain further data in regard to the amount of nutrients required for milk production when other factors for which food may be used are controlled. The special object in view was to so select the cows used that it would be possible to secure data bearing on the question of the relation of the richness of the milk to the amount of nutrients needed. The figures below show the average value in therms per pound of milk produced by each of the 10 cows used in the investigation.

[1]Research Bulletin No. 5, Missouri Exp. Station.
[2]Armsby, Farmers' Bulletin 346, U. S. Dept. Agr.

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Cow No.	Therms
206	.276
304	.304
400	.313
43	.352
62	.383
4	.388
27	.392
63	.420
303	.307
211	.273

These figures show that the milk of No. 63 had an energy value of a little over 50% more per lb. than that of No. 206. It is reasonable to assume that the nutrients required per pound of milk will therefore depend upon the quality as well as the quantity of the milk. The nutrients received by the animal must supply protein and furnish sufficient energy in the form of carbohydrates and fats.

Protein Requirements. It was not the purpose of this investigation to study the amount of protein best adapted to the production of milk. The protein requirement is discussed here merely to show, first, that moderate variations in the protein content of a ration have no marked influence upon milk production provided the allowance is above the minimum requirement of the animals, and, second, that the rations used in this investigation contained protein sufficient for all purposes for which it is intended. The Wolff-Lehmann standard prescribes 1.6 lbs. to 3.3 lbs. digestible protein for a 1000-lb. cow yielding 11 to 27½ lbs. milk per day. Haecker has demonstrated by feeding 12 cows for 154 days on two-thirds the amount of protein prescribed by this standard that it is possible for milk production to proceed with considerable less than the amount given in the standard referred to. Humphrey and Woll[1] suggest that the digestible protein may profitably range from 2 lbs. to 2.4 lbs. per day. According to Haecker [2]a cow weighing 850 lbs. and yielding 40 lbs. of 4% milk per day required 2.46 lbs. digestible protein. A 1200-lb. cow producing the same amount requires 2.71 lbs; while an 850-lb. cow producing 20 lbs. per day would use but 1.52 lbs. The ration which was used in the investigations reported supplied as much protein as was required by any of these standards as is shown in detail in a later paragraph.

[1]Humphrey and Woll, 21st Annual Report, Wis. Exp. Station (1904), p. 67.
[2]Haecker, T. L. Bulletin 79, Minn. Exp. Station.

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Animals Used. The animals used were all registered animals of the breeds represented and were not especially selected on account of large production. It has already been shown by work of this Station that when the richness of the milk is the same the nutrients required for production after maintenance has been supplied are in the same proportion as the production of the animals.[1] All the cows selected were of mature age, healthy, and at the beginning of the milking period. Data concerning the age and previous milk production of each cow is given below:

DATA CONCERNING COWS USED.

Herd No.	Breed	Age		Number lactation periods	Average yield Milk	Average per cent fat	Average yield fat
		Yrs.	Mo.				
206	Holstein	8	6	6	9,763	3.15	308
304	Ayrshire	4	6	3	6,582	3.96	261
400	Shorthorn	7	8	4	4,694	4.20	197
43	Jersey	5	1	3	6,812	4.82	329
62	Jersey	4	6	4	2,503	4.86	122
4	Jersey	7	4	5	6,151	5.04	310
27	Jersey	5	1	5	7,810	5.31	415
63	Jersey	4	7	3	4,909	5.86	288
303	Ayrshire	4	7	3	6,843	3.98	273
211	Holstein	7	0	4	12,665	3.06	388

The two given last in the table are those for which the data presented covers less than one year.

Ration Used. The cows all received the same character of ration, i. e., it was composed of the same kind and quantity of feeds mixed in the same proportions. The ratio between the silage and hay was kept as nearly uniform as possible. The roughage consisted of alfalfa hay of the grade known as "choice" and corn silage. For the first five animals put into the experiment green feed was substituted for the silage during the summer months. The last three had only alfalfa for roughness during the middle of the summer. These changes were necessary on account of it not being feasible to keep silage in condition for feeding with the few animals used.

The grain ration consisted of a mixture composed of corn meal, 4 parts, wheat bran, 2 parts, and linseen oil meal, 1 part. This grain mixture was fed in exactly this proportion to all the animals throughout the entire investigation including the maintenance trials. The feeds were fed as nearly as possible in the following proportion: grain, 1 part; hay, 1 part, and silage, 4 parts. The follow-

[1]Research Bulletin No. 2, Missouri Exp. Station.

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ing table shows how closely this ration, fed in the proportions mentioned, will meet the requirements of both a heavy and light producing cow computed according to Armsby[1]. The weight of the cow is taken as 1250 pounds, the per cent of fat as 4, and the daily yield of milk as 40 pounds in the first instance and 15 pounds in the second.

	Digestible Protein	Therms
Required for 40 lbs. milk.....	2.6	19.00
Supplied in 11 lbs. grain, 11 lbs. hay, 44 lbs. silage.....	2.5	19.35
Required for 15 lbs. milk.....	1.35	11.50
Supplied in 6.5 lbs. grain 6.5 lbs. hay, 26 lbs. silage.....	1.47	11.44

With our present knowledge of the protein requirements for milk production there can be no serious objection to the above ration on this score. The quantity of feed given was regulated as stated by the live weight of the animal. The cows were fed as much as was required to support the milk flow without allowing gain or loss in body weight.

All the cows were fed and milked in the same way as far as the routine of the work was concerned. They were not all in the experiment at the same time and the milking was therefore done by different men. Careful observations were made and notes taken regarding the health of the cows, their feeding characteristics, and any conditions or happenings out of the ordinary.

Analyses of Feed. The feed was purchased in sufficient quantities to make the number of analyses as small as feasible. The silage was sampled by taking a small quantity each day from that fed and placing it in a large closed jar in which sufficient chloroform was kept to prevent decomposition. The moisture in this composite sample was determined at the end of each 10-day period. A sample for complete analysis was made by combining the dry material from several of the 10-day samples. All feed analyses were made in the Department of Agricultural Chemistry under the direction of Dr. P. F. Trowbridge.

Sampling and Analyzing the Milk. The milk was weighed at the barn immediately after being drawn and the weights recorded upon the regular milk sheets. The milker then stirred the milk with a dipper and filled a pint milk jar about two-thirds full. This

[1]Armsby, Dr. H. P., Farmers' Bulletin 346, U. S. Dept. Agr.

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covered with a paper cap and taken to the laboratory where a y composite sample was prepared by taking an aliquot part from milking. With cows Nos. 63, 4 and 43 the composite sample ed thirty days in place of ten days. Formalin was used as a rvative and the chemical analyses which were made according ual methods included fat, protein, sugar and ash.

Weights of cows. Each cow was weighed each morning after g and before receiving water. This practice was continued dur- he entire period of milk production and maintenance and has found to be the most satisfactory plan. The heaviest milkers ome weight at the beginning of the feeding period as is the case all heavy producing cows in the first few weeks after calving. loss was restored during the year so the cows completed the imental period as nearly as possible at the same weight as at the ning.

EXPERIMENTAL DATA.

Tables 1 to 8 give a record by 10-day periods of the feed received ght of the cows used during the time in milk. The same data ws Nos. 27 and 62 have already been published.[1]

Table 9 gives the total of each feed received by the individual also their average weight during the period in milk covered e experiment.

The chemical analyses of the feedstuffs given are found in s 10 and 11. The five Jersey cows were on experiment at one and the other five at a later date which accounts for the two of analyses.

Tables 12 and 13 give the amount in pounds of each feedstuff ding to lot numbers corresponding to analyses. From this t is possible to calculate the data in other forms than the one ated if desirable. This data concerning the feed covers only received by the cows while in milk and does not cover that during the maintenance trials.

The milk yields together with chemical analyses for each period und in Tables 14 to 21. This includes the date in detail for animals. The same records for Nos. 27 and 62 are already avail- n printed form.[2] The date for Nos. 63, 4 and 43 is given in 30- periods to correspond to the milk analyses, and that of all for 10-day periods. All averages relating to milk analyses

[1] search Bulletin No. 2, Missouri Exp. Station (Tables 4 and 5).
[2] search Bulletin No. 2, Missouri Exp. Station (Tables 8 and 9).

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throughout this report are true averages calculated by dividing the total weight of the constituent by the total pounds of milk.

Table 22 gives the total yield of each constituent of the milk and the average analyses. In Table 23 is found the energy value of the total milk and per pound of milk calculated by assuming the heat value of milk protein and sugar to be 1.860 therms and fat 4.218 therms per pound.[1]

TABLE 1. SUMMARY OF FEED CONSUMED BY COW No. 206.

Period No.	Date 10 days ending	Grain Lbs.	Alfalfa hay Lbs.	Silage Lbs.	Average weight o cow Lbs.
1910					
1	Dec. 1*	59	59	233	1292
2	Dec. 11	116	116	464	1295
3	Dec. 21	120	116	261	1286
4	Dec. 31	120	120	394	1286
1911					
5	Jan. 10	120	116.5	386	1284
6	Jan. 20	100	94.5	312.5	1236
7	Jan. 30	110	110	400	1292
8	Feb. 9	110	110	400	1330
9	Feb. 19	110	110	400	1324
10	Mar. 1	110	110	400	1311
11	Mar. 11	110	110	400	1340
12	Mar. 21	110	110	400	1352
13	Mar. 31	101	101	400	1349
14	Apr. 10	100	100	400	1340
15	Apr. 20	92	100	400	1364
16	Apr. 30	90	90	400	1367
17	May 10	80	83	400	1354
18	May 20	80	80	400	1344
19	May 30	80	80	369	1345
20	June 9	89	241.5	.....	1356
21	June 19	90	250	.....	1341
22	June 29	90	250	.....	1348
23	July 9	73	213	.....	1339
24	July 19	80.5	221	.....	1304
25	July 29	85	227.9	.....	1302
26	Aug. 8	85	228	.....	1301
27	Aug. 18	80.5	222	.....	1306
28	Aug. 28	85	230	.....	1299
29	Sept. 7	85	230	.....	1298
30	Sept. 17	85	230	.....	1322
31	Sept. 27	85	230	.....	1329
32	Oct. 7	80	230	.....	1319
33	Oct. 17	73.6	214	.....	1325
34	Oct. 27	70.4	210	.....	1313
35	Nov. 6	70	177	44	1313
36	Nov. 16	70	95	203	1284
37	Nov. 26	70	70	280	1299
Total		3365.0	5685.4	7946.5	Av. 1319

* 5 Days.

[1]Armsby, Principles of Animal Nutrition, p. 279.

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ABLE 2. SUMMARY OF FEED CONSUMED BY COW NO. 304.

riod	Date 10 days ending	Grain Lbs.	Alfalfa hay Lbs.	Silage Lbs.	Average weight of cow Lbs.
1911					
1	Jan. 20	99	95.5	304	976
2	Jan. 30	105	97	305	973
3	Feb. 9	120	90	294	995
4	Feb. 19	100	85	300	972
5	Mar. 1	97.5	90	265	973
6	Mar. 11	82	90	257	959
7	Mar. 21	100	90	300	965
8	Mar. 31	100	90	300	962
9	Apr. 10	76	75.5	259	963
10	Apr. 20	69	69	283	953
11	Apr. 30	70	70	300	967
12	May 10	66.5	69	276	956
13	May 20	70	70	300	965
14	May 30	70	70	282	954
15	June 9	79	193.5	.....	982
16	June 19	80	200	.....	984
17	June 29	80	200	.....	974
18	July 9	76	194	.....	982
19	July 19	70.5	184	.....	950
20	July 29	75	200	.....	972
21	Aug. 8	78	206	.....	962
22	Aug. 18	85	215.2	.....	990
23	Aug. 28	85	218	.....	969
24	Sept. 7	85	220	.....	982
25	Sept. 17	68	201	.....	975
26	Sept. 27	85	220	.....	987
27	Oct. 7	80	220	.....	983
28	Oct. 17	75.2	206	.....	993
29	Oct. 27	70.8	200	.....	985
30	Nov. 6	69	167	44	1006
31	Nov. 16	60	88	189	982
32	Nov. 26	60	60	240	981
33	Dec. 6	60	60	240	991
34	Dec. 16	58.5	58.5	234	1005
35	Dec. 26	55	55	220	982
912					
36	Jan. 5	58.5	58.5	234	970
37	Jan. 10*	31	31	124	975
tal		2849.0	4806.7	5550	Av. 976

5 Days.

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TABLE 3. SUMMARY OF FEED CONSUMED BY COW NO. 400.

Period	Date (10 days ending)	Grain Lbs.	Alfalfa hay Lbs.	Silage Lbs.	Average weight of cow Lbs.
1910					
1	Dec. 31*	35	35	140	1134
1911					
2	Jan. 10	70	70	329	1111
3	Jan. 20	79	79	350	1120
4	Jan. 30	80	80	350	1144
5	Feb. 9	80	80	350	1150
6	Feb. 19	80	80	350	1151
7	Mar. 1	80	80	350	1162
8	Mar. 11	71	71	350	1173
9	Mar. 21	70	70	350	1170
10	Mar. 31	61	61	350	1168
11	Apr. 10	60	60	350	1174
12	Apr. 20	44	60	350	1180
13	Apr. 30	50	50	300	1154
14	May 10	50	50	300	1168
15	May 20	50	50	300	1145
16	May 30	50	50	301	1139
17	June 9	59	173.5	.....	1167
18	June 19	60	180	.....	1162
19	June 29	60	180	.....	1153
20	July 9	59	180	.....	1154
21	July 19	50	180	.....	1137
22	July 29	50	180	.....	1144
23	Aug. 8	50	180	.....	1131
24	Aug. 18	50	177.5	.....	1130
25	Aug. 28	50	179	.....	1131
26	Sept. 7	50	180	.....	1135
27	Sept. 17	50	180	.....	1125
28	Sept. 27	50	180	.....	1127
29	Oct. 7	50	164	.....	1129
30	Oct. 17	40	164	.....	1139
31	Oct. 27	36	160.8	.....	1134
32	Nov. 6	35	136	44	1142
33	Nov. 16	35	72	132	1131
34	Nov. 26	35	35	140	1130
35	Dec. 6	35	35	140	1150
36	Dec. 16	35	35	140	1135
37	Dec. 26	46.1	46.1	184.4	1113
Total		1995.1	4023.9	5950.4	Av. 1144

* 5 Days.

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TABLE 4. SUMMARY OF FEED CONSUMED BY COW NO. 43 BY 10-DAY PERIODS.

Period	Grain Lbs.	Alfalfa hay Lbs.	Silage Lbs.	Green feed Lbs.	Average weight Lbs.
1	12	40		.....	825
2	91	200	.....	.....	.....
3	120	90	300	.....	.....
4	120	90	300	.....	.....
5	120	90	300	.....	795
6	120	90	235	.....	790
7	114	90	230	.....	810
8	110	90	250	.....	809
9	106	90	270	.....	816
10	100	90	270	.....	810
11	103	90	270	.....	812
12	100	92	259	.....	793
13	100	100	277.5	.....	819
14	100	100	211.5	.....	805
15	100	100	221	.....	799
16	100	100	250	.....	806
17	100	100	231	.....	799
18	103	103	196	.....	793
19	110	110	200	.....	791.5
20	109	110	168	.....	801.5
21	100	110	185.5	.....	803
22	98	108	192.5	12	807
23	93	76	145.8	144	796
24	90	78	129.5	234	779.5
25	90	108	99.5	15	777
26	90	88	150	40	793
27	81	80	131.2	122.7	793.5
28	80	80	141.2	133.6	789.5
29	80	80	138.9	122.6	815.0
30	80	80	68.2	187.5	811.5
31	80	98	.....	219.1	812
32	80	100	.....	223.0	808
33	80	100	.....	250	809.5
34	80	100	.....	250	820.4
35	80	100	.....	250	823.7
36	68	100	.....	247.4	838
37	50	100	70.4	.....	843.5
38	16	40	94.9	.....	840
Total	3454	3591	5986.5	2450.9	Av. 807

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TABLE 5. SUMMARY OF FEED CONSUMED BY COW NO. 4 BY 10-DAY PERIODS.

Period	Grain Lbs.	Alfalfa hay Lbs.	Silage Lbs.	Green feed Lbs.	Average weight Lbs.
1	92	129	.....	.....	.....
2	98	147	.....	.....	.....
3	100	114	180	.....	805
4	100	90	300	.....	800
5	100	90	300	.....	815
6	100	90	300	.....	817
7	100	90	300	.....	815
8	100	90	300	.....	821
9	100	90	300	.....	816
10	100	90	300	.....	813
11	100	90	294.5	.....	814
12	100	90	300	.....	807
13	100	90	300	.....	819
14	100	90	300	.....	813
15	100	90	300	.....	818
16	100	90	300	.....	820
17	100	100	300	.....	815.5
18	100	100	285	.....	810
19	76	78	112	.....	804.5
20	90	92	262.5	12	807.5
21	90	66	213.5	144	810.5
22	90	64	180	216	808
23	90	105	160.5	12	800.7
24	90	88	175.3	30	808
25	81	74	187	134.5	822
26	71	80	234.2	145	839
27	70	80	217.2	128	859
28	65	80	114.8	190.8	855
29	65	98	.....	235.2	841
30	65	100	.....	242.6	843
31	65	100	.....	250	836.5
32	65	100	.....	250	832.5
33	65	100	.....	250	851
34	49.8	100	.....	250	852.5
35	36.5	100	250	.....	860.5
36	21	100	250	.....	865.5
37	.....	60	63	.....	849.5
Total	3035.3	3425	7078.8	2490.1	Av. 825

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TABLE 6. SUMMARY OF FEED CONSUMED BY COW NO. 63 BY 10-DAY PERIODS.

Period	Grain Lbs.	Alfalfa Lbs.	Silage Lbs.	Green feed Lbs.	Average weight Lbs.
1	23	60	.....	.....	960
2	120	90	300	.....	.....
3	120	90	300	.....	.....
4	106	90	300	.....	930
5	100	90	300	.....	910
6	100	90	300	.....	945
7	1000	90	300	.....	941
8	100	90	300	.....	942
9	100	90	300	.....	941
10	100	90	300	.....	951
11	91	90	300	.....	939
12	90	90	300	.....	946
13	90	90	300	.....	935
14	90	90	300	.....	939
15	90	90	300	.....	941
16	90	90	300	.....	942
17	90	90	300	.....	935
18	90	90	300	.....	947.4
19	90	90	300	.....	950.7
20	87	90	300	.....	956
21	80	86	294	12	951.5
22	80	56	216.3	144	933.5
23	80	54	180	234	919
24	80	104	156.5	15	914
25	80	80	193.5	40	936
26	80	80	206.5	137.5	941
27	80	80	207.1	145	942.5
28	80	80	228.6	126	962.0
29	68.5	80	89.6	190.1	968
30	50	98	.....	232.1	947.5
31	80	100	.....	225.6	951
32	80	100	.....	250	951.5
33	80	100	.....	250	962.5
34	54.5	100	.....	250	977.2
35	18	100	.....	250	973
36	.....	100	249.6	.....	972.5
37	21	100	250	.....	967
38	9	30	75	.....	952
Total	2968	3298	8046.7	2501.3	Av. 952

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TABLE 7. SUMMARY OF FEED CONSUMED BY COW NO
DAY PERIODS.

Period No.	Date 10 days ending	Grain Lbs.	Alfalfa hay Lbs.	Silage Lbs.
1911				
1	Jan. 30	80	80	294
2	Feb. 9	96	96	300
3	Feb. 19	100	93.5	280
4	Mar. 1	91	89	295
5	Mar. 11	90	90	300
6	Mar. 21	90	90	300
7	Mar. 31	81	81	300
8	Apr. 10	70	70	300
9	Apr. 20	54	70	340
10	Apr. 30	60	60	300
11	May 10	50	50	300
12	May 20	50	50	300
Total		912	919.5	3609

TABLE 8. SUMMARY OF FEED CONSUMED BY COW NO
DAY PERIODS.

Period No.	Date 10 days ending	Grain Lbs.	Alfalfa hay Lbs.	Silage Lbs.
1911				
1	Dec. 6	100	100	400
2	Dec. 16	100	100	400
3	Dec. 26	101.5	101.5	406
1912				
4	Jan. 5	109	109	436
5	Jan. 15	110	110	440
6	Jan. 25	115	115	460
7	Feb. 4	113.5	113.5	464
8	Feb. 14	110	110	440
9	Feb. 24	108.5	108.5	434
10	Mar. 5	106	106	424
11	Mar. 15	105	105	420
Total		1178.5	1178.5	4714.0

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TABLE 9. SUMMARY OF FEED CONSUMED.

Cow No.	Days	Grain Lbs.	Alfalfa hay Lbs.	Silage Lbs.	Green feed Lbs.	Average weight Lbs.
206	365	3365.0	5685.4	7946.5	.....	1319
304	365	2849.0	4806.7	5550.0	.....	976
400	365	1995.1	4023.9	5950.4	.....	1144
43	365	3454.0	3591.0	5986.5	2450.9	807
62	365	1907.3	1697.8	5087.5	2101.9	902
4	365	3035.3	3425.0	7078.8	2490.1	825
27	365	3424.0	2904.1	8777.9	4325.2	899
63	365	2968.0	3298.0	8046.7	2501.3	952
303	120	912.0	919.5	3609.0	.....	1020
211	110	1178.5	1178.5	4714.0	.....	1056

TABLE 10. CHEMICAL ANALYSES OF FEEDS FED NOS. 27, 62, 43, 4 and 63.

	Lot	Per cent Dry matter	Per cent Ash	Per cent Protein	Per cent Crude fibre	Per cent N. free extract	Per Cent Ether extract
Corn	1	85.04	1.19	7.97	1.90	69.99	3.99
	2	87.89	1.29	8.68	1.67	68.53	7.73
	3	90.25	1.50	8.91	1.81	72.80	5.23
Bran	1	89.51	6.67	14.55	8.49	55.06	4.75
	2	89.82	6.75	14.44	9.19	52.46	6.98
Oilmeal	1	90.89	5.36	34.36	7.98	36.45	6.74
Oats	1	89.60	3.20	11.40	10.80	59.40	4.80
Alfalfa hay	1	94.69	7.73	15.24	27.97	41.95	1.80
	2	94.16	8.25	12.69	36.35	35.33	1.56
	3	95.27	9.93	14.13	27.68	39.92	3.61
	4	94.23	8.88	15.48	28.71	37.97	3.19
	5	95.06	9.03	16.39	28.72	38.20	2.72
	6	91.09	7.87	14.75	31.60	34.80	2.07
Silage	1	20.58	1.40	1.68	5.80	11.02	69
	2	29.29	1.81	1.99	7.08	17.58	.83
	3	31.22	1.51	2.26	6.17	19.79	1.48
	4	27.56	1.69	2.41	5.14	17.28	1.04
	5	29.63	2.05	2.10	7.09	17.57	.83
	6	29.99	1.97	2.43	7.45	17.13	1.02
Green Alfalfa	1	24.54	2.63	4.63	7.27	9.60	.41
Green Clover	1	38.79	2.50	5.29	11.03	19.12	.85
Green Corn	1	27.72	1.88	2.32	7.09	15.49	.94

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TABLE 11. CHEMICAL ANALYSES OF FEEDS FED NO. 206, 400, 304, 303 and 211.

	Lot	Per cent Dry matter	Per cent Ash	Per cent Protein	Per cent Crude fibre	Per cent N. free extract	Per cent Ether extract
Corn	1	88.20	1.52	8.56	1.71	71.64	4.77
	2	87.80	1.20	8.12	2.30	72.52	3.67
	3	89.62	1.48	9.26	1.63	72.17	5.08
	4	90.04	2.40	9.33	1.88	73.68	2.76
Bran	1	90.26	7.18	15.61	10.46	51.09	5.92
	2	89.10	6.61	15.43	9.17	54.64	3.26
	3	88.42	6.93	13.98	9.70	53.97	3.85
	4	89.29	6.40	16.73	9.80	51.20	5.16
Oilmeal	1	91.49	6.26	36.40	7.98	35.34	5.51
	2	90.86	5.68	35.79	8.01	36.84	4.54
	3	90.47	6.07	34.38	7.90	35.51	6.61
	4	90.34	5.76	31.46	7.57	35.65	9.91
Alfalfa	1	90.72	8.95	14.33	28.56	35.96	2.92
	2	91.11	9.08	15.72	24.89	37.56	3.86
	3	90.78	10.06	17.09	23.49	36.47	3.67
	4	92.35	9.16	17.93	22.80	39.38	3.09
	5	91.95	6.89	14.71	27.65	40.74	1.97
	6	89.25	7.78	13.08	26.90	38.47	3.03
Silage	1	32.90	1.97	2.53	8.09	19.84	.48
	2	30.01	1.59	2.31	7.05	18.58	.47
	3	33.46	1.85	2.54	7.30	20.17	1.60
	4	32.95	2.51	2.54	7.20	19.84	.86
	5	31.86	3.88	4.41	7.48	14.38	1.71
	6	27.09	1.76	2.60	5.66	16.54	.32

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TABLE 12. AMOUNT OF FEED BY LOTS RECEIVED BY COWS NOS. 27, 62, 43, 4 and 63. (Pounds)

	Lot	No. 43	No. 62	No. 4	No. 27	No. 63
Corn	1	814.8	453.1	745.1	661.1	714.7
	2	102.8	65.3	102.8	113.1	92.6
	3	1056.0	554.3	945.8	1165.1	889.7
Bran	1	436.0	264.4	323.1	382.0	382.9
	2	550.9	292.0	495.8	607.7	465.4
Oilmeal	1	493.4	268.2	396.4	484.8	422.4
Oats	1	.....	10.0	26.0	10.0	.....
Alfalfa hay	1	402.0	157.0	258.0	126.0	222.0
	2	1050.0	691.5	1053.0	1009.5	1017.0
	3	568.0	277.6	499.0	485.0	486.0
	4	529.0	204.4	465.0	382.0	441.0
	5	902.0	289.2	902.0	661.6	902.0
	6	140.0	78.0	260.0	240.0	230.0
Silage	1	1615.0	929.5	1080.0	1620.0	1800.0
	2	1558.0	1355.0	1794.5	2112.2	1800.0
	3	1266.0	1205.4	1785.0	1972.5	1800.0
	4	722.7	734.7	878.2	1235.0	1096.7
	5	659.5	610.9	978.5	1050.6	975.3
	6	163.3	252.0	563.0	787.6	574.6
Green alfalfa	1	1084.6	1150.9	1104.3	2113.7	1132.4
Green clover	1	187.5	135.0	190.8	297.4	190.1
Green corn	1	1180.8	816.0	1195.0	1914.0	1178.8

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TABLE 13. AMOUNT OF FEED BY LOTS RECEIVED BY COWS NOS 206, 304, 400, 303 and 211.

	Lot	No. 206	No. 304	No. 400	No. 303	No. 211
Corn (Lbs.)	1	1224.0	813.6	664.5	464.0	.....
	2	62.9	62.3	45.7	57.1	.....
	3	636.0	734.6	429.8	.....	234.6
	4	.....	17.7	.....	.....	438.8
Bran (Lbs.)	1	612.0	406.8	332.2	232.0	.....
	2	31.4	31.1	22.8	28.6	.....
	3	318.0	367.3	214.9	.....	117.3
	4	.....	8.8	.....	.....	219.4
Oilmeal (Lbs.)	1	345.4	238.8	189.7	116.0	.....
	2	15.7	15.6	11.4	14.3	.....
	3	119.6	148.3	83.9	.....	58.6
	4	.....	4.4	.....	.....	109.7
Alfalfa hay (Lbs.)	1	291.0	.....	.....	.....	.....
	2	1631.0	1159.5	1029.5	819.5	.....
	3	110.0	85.0	80.0	100.0	.....
	4	904.0	750.0	684.0	.....	.....
	5	2748.9	2585.2	2135.3	.....	60.0
	6	.....	227.0	95.1	.....	1118.5
Silage (Lbs.)	1	2085.5	160.0	644.0	.....	.....
	2	1925.0	1387.0	1715.0	1289.0	.....
	3	1640.0	1170.0	1435.0	1245.0	.....
	4	1769.0	1308.0	1376.0	1075.0	.....
	5	527.0	881.0	554.0	.....	680.0
	6	.....	644.0	226.4	.....	4034.0

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TABLE 14. YIELD AND AVERAGE COMPOSITION OF MILK BY 10-DAY PERIODS. COW NO. 206.

Period	Pounds milk	Per cent fat	Per cent nitrogen	Per cent protein (N x 6.38)	Per cent sugar	Per cent ash
1	217.4*	3.27	.47	3.03	4.25	.....
2	402.6	3.71	.46	2.94	4.21	.907
3	427.3	3.55	.46	2.96	4.14	.758
4	432.8	3.31	.47	2.97	4.50	.781
5	409.2	3.30	.41	2.61	4.58	.755
6	372.6	3.26	.45	2.86	4.41	.748
7	445.4	3.35	.44	2.82	4.06	.737
8	403.3	3.44	.45	2.89	4.35	.827
9	427.5	3.60	.45	2.88	4.58	.745
10	403.1	3.60	.43	2.77	4.47	.719
11	412.8	3.56	.43	2.76	4.08	.731
12	410.0	3.95	.45	2.90	4.00	.732
13	360.0	3.80	.47	3.03	3.91	.711
14	377.4	3.80	.46	2.96	4.06	.735
15	372.1	3.70	.46	2.96	4.20	.727
16	349.0	3.81	.47	2.97	4.16	.713
17	343.2	3.56	.46	2.95	4.19	.739
18	331.1	3.86	.44	2.97	3.84	.722
19	326.4	3.55	.44	2.83	4.30	.741
20	330.6	3.10	.46	2.94	4.30	.773
21	356.3	2.70	.47	3.00	4.19	.774
22	331.9	2.81	.45	2.88	4.18	.746
23	293.1	2.65	.45	2.88	4.30	.705
24	325.0	2.87	.47	2.98	4.27	.709
25	313.7	2.91	.45	2.87	4.29	.724
26	297.3	3.49	.46	2.95	4.27	.709
27	257.1	3.40	.47	2.98	4.15	.723
28	279.5	3.33	.50	3.17	4.25	.716
29	282.2	3.14	.50	3.16	4.35	.717
30	269.3	2.93	.49	3.05	4.08	.759
31	245.7	3.16	.50	3.20	4.20	.699
32	234.4	3.39	.51	3.23	3.77	.741
33	188.8	3.21	.50	3.20	3.70	.749
34	202.8	3.22	.51	3.26	3.49	.746
35	197.8	3.57	.51	3.26	3.53	.726
36	169.9	3.93	51	3.26	2.82	.758
37	188.0	3.52	.49	3.15	4.14	.729
Average		3.401	.463	2.951	4.175	.745

* 5 Days.

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TABLE 15. YIELD AND AVERAGE COMPOSITION OF MILK BY 10-DAY PERIODS. COW NO. 304.

Period	Pounds milk	Per cent fat	Per cent nitrogen	Per cent protein N x 6.38	Per cent sugar	Per cent ash
1	366.8	4.18	.52	3.30	4.14	.761
2	389.2	4.00	.48	3.07	4.25	.762
3	397.6	4.10	.50	3.21	4.60	.726
4	388.2	3.95	.47	2.98	4.62	.709
5	344.9	3.48	.43	2.76	4.50	.748
6	326.5	3.59	.46	2.96	4.10	.690
7	336.9	3.69	.49	3.10	4.25	.711
8	322.1	3.80	.51	3.24	4.38	.731
9	260.3	4.10	.52	3.34	4.29	.730
10	276.8	3.43	.50	3.18	4.25	.726
11	264.2	3.90	.49	3.12	4.09	.729
12	261.5	3.60	.49	3.12	4.43	.716
13	268.0	3.55	.48	3.05	4.40	.713
14	271.6	3.50	.48	3.03	4.36	.760
15	275.8	3.44	.48	3.05	4.29	.759
16	282.5	3.46	.49	3.12	4.34	.724
17	263.3	3.45	.49	3.13	4.40	.719
18	253.4	3.49	.50	3.18	4.36	.708
19	262.2	3.36	.50	3.18	4.57	.670
20	262.9	3.42	.50	3.17	4.45	.710
21	260.4	3.50	.49	3.15	4.49	.719
22	246.3	4.01	.52	3.31	4.50	.682
23	262.6	3.96	.54	3.43	4.56	.707
24	242.4	4.00	.53	3.21	4.54	.694
25	233.9	3.70	.53	3.37	4.62	.699
26	225.4	4.02	.55	3.52	4.35	.747
27	215.8	3.96	.59	3.74	4.20	.751
28	180.8	4.18	.60	3.82	4.00	.748
29	173.7	4.10	.62	3.94	3.47	.700
30	145.8	4.65	.65	4.12	3.50	.764
31	141.3	4.71	.61	3.87	4.25	.740
32	132.6	5.07	.64	4.06	4.13	.769
33	132.0	5.01	.61	3.92	4.24	.778
34	143.4	4.26	.59	3.74	4.43	.737
35	142.3	4.31	.58	3.69	4.49	.769
36	137.2	4.48	.61	3.87	4.49	.739
37	78.4*	4.75	.58	3.71	4.50	.721
Average		3.853	.514	3.278	4.339	.725

* 4 Days.

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TABLE 16. YIELD AND AVERAGE COMPOSITION OF MILK BY 10-DAY PERIODS. COW NO. 400

Period	Pounds milk	Per cent fat	Per cent nitrogen	Per cent protein N x 6.38	Per cent sugar	Per cent ash
1	105.7*	4.35	.61	3.86	4.68	.801
2	207.7	4.29	.56	3.59	4.80	.791
3	213.0	4.21	.57	3.65	4.62	.832
4	228.4	3.90	.54	3.48	4.70	.800
5	228.8	3.98	.56	3.59	4.70	.811
6	227.6	3.85	.56	3.58	4.80	.801
7	224.6	3.68	.56	3.55	5.00	.817
8	225.5	3.42	.55	3.52	3.50	.795
9	220.1	3.90	.53	3.38	4.09	.806
10	207.3	3.75	.56	3.57	4.60	.765
11	199.5	4.10	.56	3.59	4.60	.766
12	199.2	3.80	.54	3.45	4.56	.767
13	192.9	3.75	.55	3.53	4.66	.769
14	190.2	3.80	.54	3.42	4.27	.781
15	177.3	3.94	.51	3.28	4.58	.762
16	164.2	4.10	.53	3.37	4.92	.763
17	163.9	3.44	.51	3.28	4.65	.773
18	166.4	3.45	.53	3.36	4.48	.744
19	155.1	3.65	.52	3.33	4.67	.763
20	166.5	3.70	.53	3.40	4.65	.760
21	163.0	3.70	.53	3.39	4.70	.745
22	158.6	3.65	.54	3.42	4.74	.749
23	156.7	3.61	.54	3.41	4.75	.743
24	142.5	4.06	.53	3.38	4.75	.738
25	142.4	3.92	.55	3.52	4.72	.748
26	134.2	4.17	.55	3.51	4.75	.783
27	114.7	3.81	.55	3.51	4.30	.791
28	93.7	4.27	.56	3.59	4.10	.799
29	96.2	3.65	.57	3.62	4.02	.778
30	80.9	4.20	.55	3.52	4.00	.806
31	74.2	4.28	.57	3.61	3.60	.799
32	71.4	4.23	.56	3.56	3.02	.789
33	60.6	4.20	.55	3.53	2.15	.793
34	57.4	4.70	.56	3.55	4.25	.785
35	56.2	4.54	.56	3.55	4.33	.807
36	51.1	4.39	.55	3.49	4.50	.792
37	55.3	4.22	.55	3.51	4.51	.789
Average		3.889	.547	3.491	4.498	.781

* 5 Days.

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TABLE 17. YIELD AND AVERAGE COMPOSITION OF MILK BY 30-DAY PERIODS. COW NO. 43.

30 days ending	Pounds milk	Per cent fat	Per cent nitrogen	Per cent protein (N x 6.38)	Per cent sugar	Per cent ash
Oct. 25	920.5[1]	4.60	.57	3.64	5.30	.710
Nov. 24	776.4	5.18	.59	3.76	5.45	.770
Dec. 24	680.0	5.42	.55	3.51	4.50	.747
Jan. 23	674.8	5.15	.52	3.32	4.55	.615
Feb. 22	652.5	5.35	.56	3.57	3.70	.707
Mar. 23	669.5	5.02	.55	3.51	4.10	.712
Apr. 22	634.9	5.20	.55	3.51	4.35	.720
May 22	615.6	5.03	.53	3.38	4.60	.728
June 21	586.8	4.80	.56	3.57	4.00	.697
July 21	619.0	4.20	.55	3.51	4.78	.664
Aug. 20	599.7	4.20	.57	3.64	4.49	.697
Sep. 23	509.8[2]	4.30	.59	3.76	3.89	.609
	8039.5	4.88	.56	3.56	4.52	.70

[1] 32 Days.
[2] 34 Days.

TABLE 18. YIELD AND AVERAGE COMPOSITION OF MILK BY 30-DAY PERIODS. COW NO. 4.

30 days ending	Pounds milk	Per cent fat	Per cent nitrogen	Per cent protein (N x 6.38)	Per cent sugar	Per cent ash
Nov. 24	1266.4[1]	5.18	.63	4.02	4.97	.842
Dec. 24	631.5	5.67	.64	4.08	3.83	.768
Jan. 23	643.6	5.80	.62	3.96	.....	.800
Feb. 22	623.2	5.95	.62	3.96	4.00	.760
Mar. 23	622.4	5.97	.65	4.15	4.20	.791
Apr. 22	524.3	6.10	.63	4.02	4.70	.792
May 22	454.3	5.72	.66	4.21	4.60	.702
June 21	454.7	5.40	.67	4.27	4.10	.661
July 21	489.9	5.17	.64	4.08	4.41	.737
Aug. 20	475.5	4.67	.65	4.15	4.36	.684
Sep. 19	426.1	5.00	.68	4.34	4.16	.732
Oct. 5	161.7[2]	5.20	.70	4.47	4.36	.731
	6773.6	5.50	.64	4.12	4.28	.76

[1] 48 Days.
[2] 16 Days.

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TABLE 19. YIELD AND AVERAGE COMPOSITION OF MILK BY 30-DAY PERIODS. COW NO. 63.

30 days ending	Pounds milk	Per cent fat	Per cent nitrogen	Per cent protein (N x 6.38)	Per cent sugar	Per cent ash
Oct. 25	500.2[1]	5.90	.69	4.40	4.70	.778
Nov. 24	641.4	6.22	.72	4.59	3.83	.780
Dec. 24	579.0	6.50	.69	4.40	.....	.741
Jan. 23	563.3	6.40	.67	4.27	4.08	.740
Feb. 22	523.6	6.43	.70	4.47	4.35	.741
Mar. 23	520.9	6.35	.70	4.47	4.60	.771
Apr. 22	590.0	6.60	.70	4.47	5.05	.795
May 22	463.3	6.28	.68	4.34	4.50	.744
June 21	470.6	5.77	.65	4.15	4.40	.713
July 21	419.9	5.57	.68	4.34	4.10	.707
Aug 20	420.1	5.20	.66	4.21	4.53	.684
Sep. 19	323.0	5.40	.69	4.40	3.66	.703
Oct. 2	118.6[2]	5.56	.67	4.27	4.28	.696
	6033.9	6.09	.68	4.37	4.21	.74

[1] 22 Days.
[2] 13 Days.

TABLE 20. YIELD AND AVERAGE COMPOSITION OF MILK BY 10 DAY PERIODS. COW NO. 303.

Period	Pounds milk	Per cent fat	Per cent nitrogen	Per cent protein (N x 6.38)	Per cent sugar	Per cent ash
1	294.9	3.73	.48	3.09	4.75	.725
2	307.5	3.75	.49	3.12	4.76	.716
3	302.9	3.62	.50	3.18	5.00	.691
4	293.4	3.56	.48	3.06	5.02	.726
5	297.2	3.97	.48	3.04	4.78	.752
6	297.2	3.86	.48	3.04	4.56	.708
7	284.3	3.78	.48	3.05	4.62	.733
8	270.8	4.10	.48	3.05	4.56	.665
9	264.3	4.13	.47	2.97	4.51	.682
10	254.2	4.04	.46	2.92	4.63	.649
11	239.9	4.02	.47	2.97	4.75	.720
12	218.5	3.89	.46	2.92	4.25	.654
Average		3.861	.476	3.040	4.695	.703

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TABLE 21. YIELD AND AVERAGE COMPOSITION OF MILK BY 10-DAY PERIODS. COW NO. 211.

Period	Pounds milk	Per cent fat	Per cent nitrogen	Per cent protein (N x 6.38)	Per cent sugar	Per cent ash
1	395.8	3.16	.47	3.02	4.57	.681
2	386.3	2.91	.45	2.89	4.72	.708
3	371.4	2.96	.43	2.71	4.80	.674
4	379.9	3.03	.44	2.83	4.82	.678
5	403.5	3.18	.44	2.82	4.86	.680
6	418.7	3.15	.45	2.84	4.95	.666
7	423.5	3.13	.45	2.87	5.05	.651
8	415.1	3.07	.46	2.96	5.05	.657
9	408.7	3.00	.45	2.89	5.05	.653
10	392.7	3.02	.45	2.89	5.09	.657
11	387.8	3.02	.45	2.89	5.08	.658
Average		3.052	.446	2.868	4.904	.667

TABLE 22. SUMMARY OF MILK YIELDS AND AVERAGE COMPOSITION.

Cow No.	Breed	Pounds milk	Per cent fat	Per cent protein	Per cent sugar	Per cent ash
206	Holstein	11,986.9	3.40	2.95	4.18	.75
304	Ayrshire	9,169.0	3.85	3.28	4.34	.73
400	Shorthorn	5,573.0	3.89	3.49	4.50	.78
43	Jersey	8,039.5	4.88	3.56	4.52	.70
62	Jersey	3,188.9	5.31	3.99	4.52	.75
4	Jersey	6,773.6	5.50	4.12	4.28	.76
27	Jersey	8,522.9	5.51	3.98	4.60	.73
63	Jersey	6,033.9	6.09	4.37	4.21	.74
303	Ayrshire	3,325.1	3.86	3.04	4.70	.70
211	Holstein	4,393.4	3.05	2.87	4.90	.67

TABLE 23. YIELD OF MILK, MILK CONSTITUENTS AND ENERGY VALUE OF MILK.

Cow No.	Pounds fat	Pounds protein	Pounds sugar	Energy value (therms)	Energy value per lb. milk (therms.)
206	407.7	353.8	499.9	3307.6	.2759
304	353.3	300.6	397.9	2789.4	.3042
400	216.9	194.5	250.7	1743.0	.3127
43	387.9	282.5	359.7	2830.7	.3520
62	169.4	127.3	144.4	1219.9	.3825
4	372.9	278.3	290.2	2630.3	.3883
27	470.0	339.4	392.6	3344.0	.3923
63	367.9	263.8	265.4	2536.1	.4203
303	128.4	101.1	156.1	1020.0	.3067
211	134.1	126.0	215.5	1200.8	.2733

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MAINTENANCE

The results of the maintenance trials for the four Jersey cows, Nos. 27, 63, 43, 62 have already been published in detail[1] and will not be repeated here. Trials were conducted in a similar manner for Nos. 206, 304, 400. At the end of the year in milk these cows were dryed as soon as possible and the maintenance trial begun about one month later or as soon as the ration could be properly adjusted to the smaller requirements. With all seven animals the ration used on maintenance was the same and consisted of the same feeds as fed when in milk. The grain was a mixture of corn meal, 4 parts, wheat bran, 2 parts and linseed oilmeal, 1 part. The ratio between the amounts of grain, hay and silage fed, was 1:1:4.

No. 206 was a large framed cow carrying but little flesh. The results seem to indicate that a cow of this type requires somewhat more than the Armsby standard for maintenance while a cow carrying more flesh is liable to come close to this standard. Probably had No. 206 carried 100 lbs. more flesh her maintenance requirement would have been closer to the standard. Such an addition to a cow of this size would not have increased the body surface to any extent and would not have been sufficient to have burdened the organs of the body.

No. 206 was started at an average weight of 1283 lbs. for the first 10 days, while the average for the last 10 days was 1303 lbs., a gain apparently of 20 lbs. in 150 days.

No. 400 averaged 1139 lbs. for the first ten days and closed at 1127 lbs., an apparent loss of 12 lbs. in 150 days. Her maintenance requirement was exceedingly close to Armsby's standard.

No. 304 average 990 lbs. for the first 10 days and 978 for the last 10, an apparent loss of 12 lbs. Her maintenance requirement was also almost exactly as called for by the standard used.

The data in Table 24 gives the average ration per day, for each of the seven cows for which maintenance trials were made, the requirement in energy value for an animal of that weight according to Armsby's standard and the energy value of the ration actually required as calculated by using Armsby's "Production Value" tables.

Table 25 gives the total amount of protein and energy value required for the entire maintenance period calculated by using Armsby's standard.

[1]Research Bulletin No. 5, Missouri Exp. Station.

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TABLE 24. SUMMARY OF RATION FED ON MAINTENA

Cow No.	Days on maintenance	Pounds grain per day	Pounds hay per day	Pounds silage per day	Weight of animal	Armsby standard (therms)
206	150	4.48	4.48	17.94	1291	7.25
304	130	3.32	3.32	13.28	1984	5.90
400	150	3.77	3.77	15.06	1129	6.58
27	160	3.29	3.30	13.20	889	5.55
62	180	2.92	2.92	11.76	916	5.59
63	120	2.95	2.95	11.88	886	5.80
4	150	3.43	3.43	13.78	792	5.20

TABLE 25. TOTAL PROTEIN AND ENERGY REQUIRED MAINTENANCE.*

Cow No.	Therms per day	Therms for entire period	Pounds protein per day	Pounds protein fo entire peri
206	7.25	2646.2	.61	222.7
304	5.90	2153.5	.49	178.9
400	6.58	2401.7	.56	204.4
43	5.19	1894.3	.42	153.3
62	5.59	2040.3	.46	167.9
4	5.20	1898.0	.43	157.0
27	5.55	2025.7	.46	167.9
63	5.80	2117.0	.48	175.2
303	6.10	732.0	.51	61.2
211	6.22	684.2	.52	57.2

* Calculated by using Armsby's Standard.

PROTEIN AND ENERGY USED FOR MILK PRODU

Comparison With Armsby's Standard. As previousl no attempt is made in this investigation to determine th requirement for milk production. The general plan was sufficient protein and study the requirement for energ Table 26 therefore is introduced to show how much pr used per pound of milk by each cow with no intention of it to be the proper amount. This table is calculated quantity of feed given each animal as found in Table 9 using Armsby's "Production Value" table which is of cou upon average composition of feedstuffs. Later in Table 3 protein figure is given based upon actual analyses and

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TABLE 26. PROTEIN IN FEED FOR MAINTENANCE, FOR PRODUCTION, AND PER POUND OF MILK.

Cow No.	Total protein consumed Pounds	Total protein for maintenance Pounds	Protein available for production Pounds	Protein per pound of milk Pounds
206	824.9	222.7	602.3	.0502
304	687.7	178.9	508.8	.0554
400	543.3	204.4	338.9	.0608
43	708.1	153.3	554.8	.0690
62	402.1	167.9	234.2	.0734
4	662.0	157.0	505.0	.0745
27	713.1	167.9	545.2	.0640
63	655.2	175.2	480.0	.0795
303	193.4	61.2	132.2	.0397
211	249.7	57.2	192.5	.0438

coefficients. It will be observed that the amount of protein used by the cows No. 400, 304, and 303 that produced milk with a fat content of close to 4% was higher in two cases and lower in one than the amount suggested by Armsby. Since Armsby does not give the protein requirements for cows with milk richer than 4% the comparison cannot be carried farther.

The main comparison to be made is with the total energy value of the ration. Table 27 is calculated by using the amount of each feedstuff as given in Table 9 and the "Production Value" tables of

TABLE 27. ENERGY IN FEED FOR MAINTENANCE, FOR PRODUCTION, AND PER POUND OF MILK.

Cow No.	Energy value of feed (Therms)	Energy for maintenance (Therms)	Energy available for production (Therms)	Energy used per pound milk (Therms)
206	5823.6	2646.2	3177.4	.265
304	4733.2	2153.5	2579.7	.281
400	3882.9	2401.7	1481.2	.266
43	5159.1	1894.3	3264.8	.405
62	3139.4	2040.3	1099.1	.345
4	4968.9	1898.0	3070.9	.453
27	5598.3	2025.7	3572.6	.419
63	5036.1	2117.0	2919.1	.483
303	1605.5	732.0	873.5	.263
211	2079.7	684.2	1395.5	.317

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Armsby. Since this table is based upon average analyses it that in Table 27 no use is made of the actual chemical a which were made of all the feeds used. The "Production table is used in this case as it is intended to be used by as all feeds to be of average composition.

The "energy for maintenance" is the amount that each would require for this purpose during the entire period in assuming that their maintenance requirement was that of Ar standard for an animal of the respective weights as calcula Table 24. The "energy available for production" is the dif between the total energy value of the ration and the amount for maintenance. The available energy value divided by th pounds of milk produced by the animal gives the final figu energy value used per pound of milk. It will be observed th requirement varies from .265 therms for No. 206 with an a fat content of 3.40, to .483 therms for No. 63 with 6.09% fat figures for No. 303 and 211, the two that were in the experim less than a year compare fairly well with the others except N is considerably higher than some others giving richer milk. results will be discussed further in another connection but it dent from the data given that the richness of the milk is an imp factor in determining the amount of feed required and that quirement varies quite consistently with the energy value milk.

Table 28 brings together for comparison the average per fat for each cow, the energy required per pound of milk calcul

TABLE 28. ENERGY VALUE OF FEED PER POUND OF M

Cow No.	Per cent fat in milk	Armsby's standard: Therms per pound of milk	Actual maintenance: Therms per pound of milk	Energy per po mi (The
206	3.40	.265	.245	.27
304	3.85	.281	.283	.30
400	3.89	.266	.261	.31
43	4.88	.406	.....	.35
62	5.31	.345	.372	.38
4	5.50	.453	.409	.38
27	5.51	.419	.437	.39
63	6.09	.483	.524	.42
303	3.86	.263	.....	.30
211	3.05	.317	.....	.27

* Calculated by Armsby's Standard and Tables and by Actual Mainten Armsby's Tables.

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in Table 27 and the energy required per pound of milk when the actual maintenance requirements are used in place of using Armsby's standard.

No. 206, for example, according to the Armsby standard should have a maintenance requirement of 7.25 therms per day while the result of the 150-day trial indicated that she used 7.91 therms. The latter figure is used in making the calculation given in the column headed "actual maintenance" in Table 28. The calculations are made by using the same "Production Value" figures. The figures obtained in this manner are necessarily more accurate since one varying factor, that of maintenance requirement, is eliminated by using the actual in place of the estimated.

The cows for which no figures are given in the column headed "Actual Maintenance" are those for which maintenance trials were not made. The energy required per pound of milk as calculated in this manner varies from the first calculation with certain animals and tends to make the results more consistent. For example No. 62 which shows a very low energy requirement per pound of milk calculated by using Armsby's standard for maintenance shows a considerably higher figure on account of having an exceedingly low maintenance figure. In general, the data presented shows that for those cows, No. 304, 400, 303, that produced milk containing close to 4% that the requirements were not far from the standard as suggested by Armsby. Three of these cows while producing milk showing between 3.80 and 3.90% fat used about .03 therms below Armsby's figures for milk with 4.0% fat. It should be borne in mind that these cows were farrow and kept at uniform weight, conditions that do not necessarily exist with many cows in ordinary dairy herds. Armsby gives no figures for the requirements of cows producing above 4% fat, therefore no comparison can be made of the energy used by the cows producing the richer milk.

COMPARISON WITH HAECKER'S STANDARD.

Table 29 gives the amount of digestible protein, carbohydrates and fat that each cow would require per pound of milk according to Haecker's standard. The column headed "Total Nutrients" is introduced to combine the nutrients so a comparison may be made with the amount actually received. In calculating these figures the digestible ether extract was multiplied by 2.25 and the result added to the digestible protein and carbohydrates.

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TABLE 29. NUTRIENTS REQUIRED FOR MILK BY HAECKER'S STANDARD.*

Cow No	Per cent fat	Protein	Carbo-hydrates	Ether extract	Total Nutrients
206	3.40	.043	.194	.015	.271
304	3.85	.046	.210	.016	.292
400	3.89	.046	.210	.016	.292
43	4.88	.053	.243	.018	.337
62	5.31	.056	.256	.019	.355
4	5.50	.057	.263	.019	.363
27	5.51	.057	.263	.019	.363
63	6.09	.061	.283	.021	.391
303	3.86	.046	.210	.016	.292
211	3.05	.040	.184	.014	.256

* Maintenance calculated by Haecker's Standard.

Table 30 is calculated to show the nutrients actually used by the cows in this experiment. In making these calculations the total digestible nutrients were found by applying to the feed received the figures from the tables found in Henry's "Feeds and Feeding."

TABLE. 30. NUTRIENTS USED FOR MILK PRODUCTION.

Cow No.	Protein	Carbo-hydrates	Ether extract	Total nutrients	Haecker's maintenance. Percent above standard	Actual maintenance. Percent above standard
206	.068	.152	.015	.254	—6.3	+11.0
304	.077	.189	.016	.302	+3.4	+19.8
400	.086	.105	.019	.234	—19.9	+17.5
43	.093	.296	.023	.441	+30.8	.....
62	.099	.119	.034	.295	—16.9	+27.8
4	.103	.325	.026	.589	+62.2	+33.3
27	.086	.292	.024	.432	+19.0	+23.1
63	.108	.319	.029	.492	+25.8	+55.2
303	.053	.154	.016	.243	—16.8	.....
211	.059	.219	.017	.316	+23.4	.....

The amount to be deducted for maintenance was determined by using Haecker's standard of digestible nutrients, namely, protein, .7 lbs., carbohydrates, 7.0 lbs., ether extract, .1 lb. per 1000 lbs. live weight. It is impossible to compare the different nutrients di-

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rectly to any advantage since the cows in this trial received so much more protein in their ration. The only comparison of much significance is between the columns giving total nutrients. The column next to the last in this table gives the per cent of total nutrients above or below that called for by Haecker's standard for a cow giving milk of this particular richness. It will be observed that five out of the eight cows whose records cover the entire year used more nutrients than Haecker's standard calls for. In one case the excess was 62.2%. Three of the eight cows used less than Haecker's standard calls for. Of the two cows used for shorter intervals one used less and one more than the standard. Only one of the cows producing milk with over 4% of fat was able to do so with the nutrients called for by this standard. The last column is calculated by using the actual maintenance requirements as found for the animals for which the figures are given. The average amount of grain, hay and silage fed as given in Table 24 was used with the same figures as before for calculating the digestible nutrients. The results calculated in this way are far more consistent. In every case the cows used more than the amount prescribed by Haecker's standards. It should be further noted that this excess is greater with the richer milk. At a later point data is introduced for two Jersey cows producing milk testing from 6.47% to 7.27% fat and covering a period of 142 days. One producing 2238 lbs. of milk with a fat content of 7.27%, used nutrients to the amount of 27.2% above the standard. The other producing 1942 lbs. milk with 6.47% fat used 10.2% excess of nutrients. Maintenance was assumed to be as calculated from Haecker's standard.

The only conclusion that can be drawn from the data given is that Haecker's standard did not, for these cows, supply sufficient nutrients for milk production and maintenance. The deficiency was most marked with those cows producing rich milk. Haecker's experiments upon which he based his standard covered 154 days, during the winter season. Only two cows were included that produced milk with a fat content above 5% and these were not far in excess of that figure. He assumed average maintenance requirements. His low results may have come about from having used cows with maintenance requirements below the average which would tend to lower the amount used per pound of milk produced. It is also possible that his cows were underfed but the time was not sufficient to make the condition noticeable.

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REQUIREMENTS FOR PRODUCTION

As already indicated all feeds given were subjected to chemical analyses. The results of these analyses together with the amount of each lot consumed by each cow during the production period are found in Tables 10, 11, 12 and 13.

Results of Digestion Trials. A digestion trial was also conducted with five of the animals, namely, No. 206, 304, 400, 62 and 27, covering 10 days when near the maximum milk production, and again for each of the same animals when dry and on a maintenance ration. This data for Nos. 27 and 62[1] has already been published. As a result of this work it has been found that each of the five animals showed a digestion coefficient when on full feed that was considerably lower than the average digestion coefficients in common use. In making these calculations the figures given by Jordan[2] found in Table 31 were taken as average digestion coefficients. In Table 32 the lines headed "Average" give the digestion coefficient for each cow calculated for each constituent of the ration using the chemical analyses and the average digestion coefficients. The lines marked "Actual" give the per cent of that constituent of the ration as actually digested according to the results of the 10-day trial.

It will be observed that in only a single case was the digestion of any of the constituents equal to what it should be calculated by average figures generally used. It should be said further that the

TABLE 31. AVERAGE DIGESTION COEFFICIENTS USED.

	Protein	Crude fibre	Nitrogen-free extract	Ether extract
Corn	67.9	58.0	94.6	92.1
Bran	77.8	28.6	69.4	68.0
Oilmeal	88.8	57.0	77.6	88.6
Alfalfa	72.0	46.0	69.2	51.0
Silage	49.3	66.7	68.6	80.0
Green alfalfa	74.0	43.0	72.0	39.0
Green Corn	54.0	51.0	75.0	78.0
Green Clover	66.0	49.0	71.0	61.0
Oats	77.0	31.0	77.0	89.0

[1] Research Bulletin No. 4, Missouri Exp. Station.
[2] Jordan, W. H., The Feeding of Animals.

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TABLE 32. COMPARISON OF AVERAGE AND ACTUAL DIGESTION COEFFICIENTS. FULL RATION.

	Cow No. 206. Holstein	Cow No. 400. Shorthorn	Cow No. 304. Ayrshire	Cow No. 27. Jersey	Cow No. 62. Jersey
Total dry matter					
Average	70.46	70.19	71.78	70.81	70.79
Actual	65.34	65.52	64.31	66.27	66.95
Protein					
Average	69.52	68.68	70.82	70.20	70.20
Actual	62.48	61.30	61.57	58.75	60.58
Crude fibre					
Average	55.47	56.46	54.62	53.90	53.90
Actual	50.41	50.94	47.48	53.82	53.89
Nitrogen free extract					
Average	75.57	75.04	77.08	76.60	76.60
Actual	72.54	72.28	71.39	72.62	73.62
Ether extract					
Average	70.44	70.79	72.11	78.00	78.00
Actual	68.72	71.80	72.00	66.95	59.82

same cows on maintenance showed a digestion coefficient even higher than average figures as may be seen from Table 37. The next step was to calculate the actual digestion coefficients in per cent of the average coefficients. The results of this calculation are given in Table 33. For example, No. 27 according to this could be assumed

TABLE 33. ACTUAL DIGESTION COEFFICIENTS EXPRESSED IN PER CENT OF AVERAGE COEFFICIENTS.

Cow No.	Protein	Crude fibre	Nitrogen-free extract	Ether extract
27	83.69	100.00	94.84	85.83
62	86.30	100.00	96.11	76.69
206	89.88	90.88	95.98	97.56
304	86.96	86.93	92.63	100.00
400	89.69	90.21	96.32	101.42

to digest 83.69% as much protein as would be found by using average digestion coefficients. In this way a table of digestion coefficients for each feedstuff was calculated for each of the animals

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TABLE 34. DIGESTION COEFFICIENTS AS CALCULATED FOR COW NO. 27.

	Protein	Crude fibre	Nitrogen-free extract	Ether extract
Corn	56.80	58.00	89.70	79.00
Bran	65.10	28.60	65.80	58.40
Oilmeal	74.30	57.00	73.60	76.00
Alfalfa hay	60.30	40.00	65.60	43.80
Silage	41.30	66.70	65.00	68.70
Green alfalfa	61.90	43.00	68.30	33.50
Green corn	45.20	51.00	71.10	67.00
Green clover	55.20	49.00	67.30	52.40
Oats	64.40	31.00	73.00	76.40

used. Table 34 gives this data for No. 27. It has to be assumed in doing this that since No. 27 digested only 83.69% of the protein from the total ration that she should have done by average figures, that the digestion coefficient for the protein was depressed an equal amount for the protein in each part of the ration. Since the rations fed were kept in nearly the same proportions as between the grain and roughness this assumption would seem to be one that could be made without serious error. The digestion coefficients were calculated for each of the other animals in precisely the same manner but these figures are not put in print. Since no digestion trials were made for Nos. 63, 4, 43, 211, 303, the coefficients used for calculating the results from these animals are the averages of those for the five animals, Nos. 206, 304, 400, 27, 62.

Table 35 gives this data in detail as calculated for No. 27. The others were prepared in the same manner but it was not thought necessary to put these figures into print in detail as they can be calculated by anyone interested from the data given. The summary is found in Table 38.

Calculation of Energy Value. The calculations based upon the chemical analyses and the digestion trials were made for each animal separately and were conducted as follows: The total amount of each constituent, as for example protein fed in the form of corn, was determined from the tables giving the record of feed consumed (Tables 1, 8) and from the record of chemical analyses (Tables 10, 12). The next step was to apply the digestion coefficient as calculated for the particular cow from the digestion trial (Table 34). This gives the amount of each digestible nutrient as far as could be calculated

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BLÆ 35. DIGESTIBLE NUTRIENTS AND ENERGY VALUES IN MILK DURING YEAR. COW NO. 27.

Feed	Digestible Nutrients			Total crude fibre	Digestible albuminoids	Energy value (Th'rms)
	Protein (Lbs.)	Carbohydrates (Lbs.)	Ether extract (Lbs.)			
rn	94.5	1266.0	75.9	35.5	83.2	1636.6
an	93.3	373.4	35.4	88.3	75.5	437.4
meal	123.8	152.1	24.8	38.7	121.0	336.0
alfa hay	252.5	1130.9	31.0	911.9	164.8	879.2
ge	76.3	1332.3	60.1	561.9	47.4	1340.9
en alfalfa	60.6	204.6	2.9	153.6	31.3	194.8
en clover	8.7	54.3	1.3	32.8	6.1	50.3
en corn	20.1	280.0	12.1	135.8	6.9	276.6
s	.7	4.7	.4	1.1	.6	6.2
Total	730.5	4798.3	243.9	1959.6	536.8	5158.0

the animal in question. The amount of digestible albuminoids d the energy value in therms was calculated according to the thod followed by Armsby in the calculation of his "Production lue" tables.[1] Under the head "Digestible Albuminoids" is cluded the digestible proteins minus the protein in the amide form. e amides are assumed to be entirely digestible. The percentage "amides" assumed for the different feeds were as follows:

Corn..... .58 per cent	Silage..... .33 per cent
Bran.....1.80 per cent	Green clover.... .86 per cent
Oilmeal..... .58 per cent	Green corn..... .69 per cent
Alfalfa hay...3.02 per cent	Green alfalfa...1.39 per cent

The first four analyses of the feedstuffs used, were made by T. E. oodward by the use of Stutzer's reagent. The figures used for the ner feedstuffs were calculated from Farmers' Bulletins 22 and 346. e digestible protein (albuminoids) as given by Armsby in Bulletin 5 is subtracted from the digestible protein in Bulletin 22. The ference represents the amides. The total digestible albuminoids ceived by each cow is also given in Table 38.

Maintenance. The calculations for maintenance were based on the chemical analyses of the feed and the digestion coefficients calculated for the individual animals. Complete data regarding e maintenance trials for Nos. 27, 62, 4 and 63 are already in print.

[1]Armsby, Bulletin 71, Pa. Exp. Station.

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The composition of the ration fed 206, 304 and 400 may be calculated by taking the amount fed per day from Table 24 and using the chemical analyses as given in Table 36. Table 37 is made up in the

TABLE 36. COMPOSITION OF FEED GIVEN COWS NOS. 206, 400 AND 304 ON MAINTENANCE.

	Percent protein	Percent crude fibre	Percent nitrogen free extract	Percent ether extract	Percent ash
Corn	9.33	1.88	73.68	2.76	2.40
Bran	16.73	9.80	51.20	5.16	6.40
Oilmeal	31.46	7.57	35.65	9.91	5.76
Alfalfa	13.08	26.90	38.47	3.03	7.78
Silage	2.81	6.12	17.81	0.34	2.12

TABLE 37. COMPARISON OF AVERAGE AND ACTUAL DIGESTION COEFFICIENTS ON MAINTENANCE.

	Cow No. 206 Holstein	Cow No. 400. Shorthorn	Cow No. 304. Ayrshire	Cow No. 27. Jersey	Cow No. 62. Jersey
Total dry matter					
Average	65.3	65.3	65.3	69.1	69.7
Actual	70.5	69.9	69.6	73.8	72.2
Protein					
Average	69.1	69.0	69.2	69.4	68.7
Actual	71.1	69.7	73.5	67.3	65.5
Crude fibre					
Average	56.3	56.3	56.2	52.7	53.8
Actual	67.8	62.8	63.4	55.3	52.1
Nitrogen free extract					
Average	75.5	75.5	75.6	74.6	75.4
Actual	79.9	77.7	79.1	82.1	81.0
Ether extract					
Average	71.8	71.8	71.9	77.0	76.7
Actual	75.7	75.1	78.1	73.2	73.9

same form as Table 32. The figures in the lines headed "Average" represent the per cent of the ration that should be digested calculated from average digestion coefficients as given in Table 31. The figures in the lines headed "Actual" show the amounts

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digested as found by the digestion trial. From this data a table of digestion coefficients on maintenance was calculated for each animal as explained for Table 34. From these tables the data was calculated which appears under the heads of maintenance in Table 38. The digestible albuminoids and the energy value were calculated as explained in the paragraph, "Calculations of Energy Value," p..—

Table 38 gives the total digestible protein, carbohydrates and ether extract calculated as explained by using the chemical analyses of the feed consumed and the digestion coefficients as calculated for each cow from the digestion trials. The digestion coefficients used for Nos. 4 and 63, however, are the averages for the five for which digestion trials were made. The maintenance data was not taken for No. 43 and for this reason only the therms for maintenance are included which are calculated from the averages of the others.

TABLE 38. NUTRIENTS AND ENERGY FOR MILK PRODUCTION.

	Digestible nutrients			Digestible albuminoids	Energy value (Therms)
	Protein	Carbohydrates	Ether extract		
Cow No. 206					
Total consumed.....	1005.0	5278.4	273.2	775.8	5956.5
For maintenance.....	442.3	2845.5	109.1	355.5	3133.9
For milk (11,986 lbs.).	562.7	2432.9	164.1	420.3	2822.6
For 1 pound milk, 3.40 percent fat..	.0469	.203	.0136	.035	.235
Cow No. 304					
Total consumed ...	807.5	4044.2	229.9	617.7	4426.2
For maintenance....	337.6	2060.4	83.2	273.8	2287.1
For milk (9169) lbs..	469.9	1983.8	146.7	343.9	2139.1
For 1 pound milk, 3.85 percent fat..	.051	.216	.016	.0375	.233
Cow No. 400					
Total consumed.....	675.3	3555.9	186.6	515.4	3885.0
For maintenance....	361.4	2301.7	88.7	289.1	2528.0
For milk (5573 lbs.)	313.9	1254.2	97.9	226.3	1357.0
For 1 pound milk, 3.89 percent fat..	.056	.225	.0175	.0406	.243
Cow No. 43					
Total consumed.....	760.5	4372.9	267.9	575.2	4822.0
For maintenance....	.....	.....	.....	.....	1894.3
For milk (8039 lbs.).	.....	.....	.....	.....	2927.7
For 1 pound milk, 4.88 percent fat..	.....	.....	.....	.....	.364

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TABLE 38. NUTRIENTS AND ENERGY FOR MILK PRODUCTION. (Continued.)

	Digestible Nutrients			Digestible albuminoids	Energy value Therms
	Protein	Carbohydrates	Ether extract		
Cow No. 62					
Total consumed.....	421.2	2719.8	119.7	312.4	2884.4
For maintenance....	267.9	1832.3	99.3	211.7	2002.8
For milk (3188 lbs.)	153.3	887.5	20.4	100.7	881.6
For 1 pound milk, 5.31 percent fat..	.048	.278	.006	.032	.276
Cow No. 4					
Total consumed.....	708.7	4318.2	257.6	528.7	4695.9
For maintenance....	309.5	2173.2	118.3	243.5	2387.0
For milk (6773 lbs.).	399.2	2145.0	139.3	285.2	2313.9
For 1 pound milk, 5.50 percent fat..	.0587	.3167	.0205	.042	.3416
Cow No. 27					
Total consumed.....	730.5	4798.3	243.9	536.8	5158.0
For maintenance....	298.6	2128.0	115.0	235.4	2330.5
For milk (8522 lbs.).	431.9	2670.3	128.9	301.4	2827.5
For 1 pound milk, 5.51p ercent fat..	.051	.313	.015	.035	.332
Cow No. 63					
Total consumed.....	707.6	4324.0	258.8	528.2	4700.3
For maintenance....	270.8	1865.5	99.3	213.8	2036.7
For milk (6033 lbs.).	436.8	2458.5	159.5	314.4	2663.6
For 1 pound milk, 6.09 percent fat..	.072	.4075	.026	.052	.4415

Energy Used for Production. The energy required per pound milk calculated from the chemical analyses and the digestion trials is lower than that given in Table 27 where the calculations were based upon Armsby's standard for maintenance and his "Production Value" tables used to estimate the amount of energy value contained in the ration. The figures also are lower than those found in Table 28 where actual maintenance was deducted and the "Production Value" tables used to calculate the value of the ration consumed. One reason why the requirements, as calculated in Table 38, are lower is that the cows in every case did not digest as high a proportion of the ration as is represented by average digestion coefficients which are made the basis of Armsby's "Production Value" tables while on maintenance the same cows digested fully as much

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as indicated by average digestion coefficients. The results of this is to decrease the apparent requirements in the way of energy value. It should be pointed out in this connection that the digestion trial carried out when the cows were in milk was made at the maximum production and since the digestion coefficient was increased when the cows were dry and on a low ration as needed for maintenance it is probable that during that part of the milking period when the production of milk was low the digestion coefficient was really higher than the one calculated. For this reason it is probable that the requirements given per pound of milk in Table 38 is really a little too low. It is recognized that the data given in Table 38 offers little that can be made use of at present in practical feeding operations.

Do Production Value Tables Apply to Milk Production? The author believes that the data presented justifies the question being raised as to the accuracy of the "Production Value" tables as prepared by Kellner and Armsby when applied to milk production. Kellner used mature fattening oxen in his investigation, and the "Production Value" tables of Armsby are based upon these results. Armsby states that practically no experiments of similar character are available for other purposes of feeding. Another point indicating that there is a discrepancy somewhere is the fact that the energy value of the milk (Table 23), with one exception, and that the cow with the richest milk is actually higher than the energy value of the feed necessary to produce it. (Table 38.) If the energy value of the feed is calculated directly by Armsby's "Production Value" tables(Table 28) six out of ten cows show more energy in the milk than in the feed. It would appear more probable that the error is in the energy value used for the feed than in the figure used for the energy value of the milk solids which presumably has been determined with reasonable accuracy. The author believes the data presented for the seven cows that were in the investigation during an entire year and for which maintenance trials were afterwards conducted is as accurate as can be taken by the means of investigation at hand. To carry the problem of the production value of feeds when used for milk production to a solution will probably require an extensive series of investigations by the use of a calorimeter with cows in milk.

DATA FROM OTHER SOURCES.

The data as presented shows that the energy requirements for very rich milk is higher in proportion to the energy value of the milk than is the requirement for milk poorer in fat. The figure for No.

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63 with an average of 6.09% fat is especially high. The following data is introduced for further comparison regarding the requirements for rich milk. Accurate feed and milk records are at hand for two Jersey cows in the University herd, but no maintenance data for these cows is available. This data is given in Table 39. The calculations are all made by using Armsby's "Production Value" tables and his maintenance standard. During this period the weights of the cows remained practically constant although both were pregnant. The energy per pound of milk is high with both. Cow No. 50 with 7.27% fat is practically the same as No. 63 reported in Table 28 that produced milk with 6.09% fat. No. 55 with a fat content of 6.47% is lower than No. 63. This bears out in general the accuracy of the data from No. 63 covering the full year but suggests the possibility that No. 63 for some reason required rather more than the average for this richness of milk.

TABLE 39. FEED AND PRODUCTION OF TWO JERSEY COWS FOR 142 DAYS.

	Cow No. 50	Cow No. 55
Alfalfa hay	956 lbs.	877 lbs.
Clover hay	378 lbs.	362 lbs.
Cowpea hay	90 lbs.	90 lbs.
Silage	3629 lbs.	3203 lbs.
Grain	1003 lbs.	730 lbs.
Weight of cow	898 lbs.	823 lbs.
Total energy in ration	1860.3 therms	1668.1 therms
Energy for maintenance	792.4 therms	782.6 therms
Energy for milk	1067.9 therms	891.4 therms
Milk produced	2238.6 lbs.	1942.6 lbs.
Average percent fat	7.27 percent	6.47 percent
Energy per pound milk	.477 therms	.458 therms

Table 40 gives results taken from the work of Savage,[1] Most of his cows made decided gains in weight and for that reason only those were selected that remained practically uniform in weight during the 142 days of the experiment. In general his figures which are likewise calculated by using Armsby's "Production Value" tables and maintenance standard, show somewhat lower range than those reported in Table 28.

[1]Savage, E. S., Bulletin 323, Cornell University Exp. Station.

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TABLE 40. REQUIREMENTS FOR MILK PRODUCTION—CORNELL UNIVERSITY HERD.

Name	Breed	Pounds milk per day	Average per cent fat	Energy per pound milk (Therms)
Hector's Buta	Jersey	23.49	5.53	.368
Cornella	Jersey	18.49	5.31	.376
Cornella	Jersey	24.12	5.28	.305
Effie	Guernsey	18.58	4.66	.333
Sigma	Holstein	35.81	3.93	.281
Charity	Holstein	31.82	3.73	.285
Omega	Holstein	25.91	3.72	.353
Psi	Holstein	23.98	3.46	.281
Chi	Holstein	27.66	3.44	.306
Eta	Holstein	36.25	3.22	.262

Relation of Richness of Milk to Economy of Fat Production. Table 41 gives the total energy in therms used by each cow per pound of fat, also the energy in therms per pound of fat after deducting maintenance. In calculating the energy available for milk production, the actual maintenance requirements as given in Table 24 are used. For No. 43, 303 and 211, for which no maintenance data was taken, it is assumed that their maintenance requirements were those called for by Armsby's standard. The total energy value of the feed is taken from Table 27. This data shows that the cheapest production of fat is with heavy producing cows, such as No. 27, that secrete milk with a high per cent of fat. This corroborates the general data along this line which indicates that cows of those breeds having a high fat content are on the average slightly more economical producers of fat. Table 41 also exhibits the well-known facts that a high production of fat is an economical one from the standpoint of feed consumed. No. 27 used 11.91 therms per pound fat while No. 62 used 18.53 therms. No. 304 produced 353 pounds of fat and used total energy to the amount of 13.4 therms per pound fat. No. 400, producing milk of similar richness but only 217 pounds in quantity, used 17.9 therms per pound fat. The last column is significant. It shows the energy required per pound of fat after deducting maintenance. It should be kept in mind that the data for Nos. 303 and 211, both of which exhibit wide variations, covers only 110 and 120 days respectively and that their maintenance is estimated. Leaving these out the data on the others shows a striking uniformity. The energy per pound of fat is less in every case for those animals producing the milk lower in per cent of fat. The Jerseys with the

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high fat content used more energy, varying from 7.39 to 8.53. The highest again is for the richest milk. The closeness of the results from No. 62 and No. 27 is striking in view of the wide variation in production. This fact has already been pointed out in another connection.[1]

TABLE 41. ENERGY REQUIRED PER POUND FAT.

Cow No.	Total energy in feed Therms	Fat produced (Pounds)	Total energy per pound fat (Therms)	Energy available for production (Therms)	Available energy per pound fat (Therms)
206	5823.6	407.7	14.28	2936.4	7.20
304	4733.2	353.3	13.40	2594.3	7.34
400	3882.9	216.9	17.90	1455.6	6.71
43	5159.1	387.9	13.30	3264.8	8.42
62	3139.4	169.4	18.53	1252.3	7.39
4	4968.9	372.9	13.32	2757.0	7.39
27	5598.3	470.0	11.91	3477.6	7.40
63	5036.1	367.9	13.69	3134.4	8.52
303	1605.5	128.4	12.50	873.5	6.80
211	2079.7	134.1	15.50	1395.5	10.40

The usual explanation of the more economical production of fat by Jerseys and Guernseys has been the fact that milk of these breeds contains more fat in proportion to the other milk constituents. The data presented strongly suggests another explanation. It is shown conclusively that after maintenance is deducted cows of the Jersey breed use more, rather than less energy value per pound of fat, and per therm of energy in the milk solids. This means that the difference is with the maintenance. The Jersey cows in our investigation, and the same would be true for the breed as a whole, produced more fat in proportion to their size than did the Shorthorn or Holstein. The food of maintenance therefore per unit of butterfat produced is less than with the other breeds which makes production of fat more economical on the basis of the total feed required. This is shown clearly by the following:

	Pounds fat	Average Weight	Maintenance therms	Per cent of ration for Maintenance.	Total Energy per pound fat.
No. 206.....	407.7	1319	2887.2	49.5	14.28
No. 27.....	470.0	899	2120.7	37.8	11.91

[1]Research Bulletin No. 2, Missouri Exp. Station.

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The total energy per pound of fat is, in this case, less for No. 27 with the greater fat production and smaller maintenance. At the same time No. 206 used her feed available above maintenance slightly more economically than did No. 27.

Relation of Richness of Milk to Economy of Production of Total Solids. It has been generally assumed that cows producing rich milk produce fat more economically and the data given indicates there is some basis for this belief although the advantage in favor of the rich milk is small. The data at hand also admits of a study of the relation of the energy in available feed to the energy value of the total solids produced in the milk. Table 42 indicates this relation. The comparison is made of the available energy, found as for Table 41, and expressing the relation of this energy to that of the milk produced.

These figures show, with the exception of No. 211, on experiment for a short period, that the highest ratio is with the milk containing the least fat. In other words a therm of energy in the feed produced more energy in milk when the per cent of fat was low than when it was high. Apparently a given amount of feed is most efficient when used to produce milk medium to low in fat. It appears from this that the production of fat is a greater tax upon the animal than is the production of other constituents of the milk carrying equal energy value.

TABLE 42. RATIO OF ENERGY IN FEED TO ENERGY IN MILK

Cow No.	In feed (Therms)	In milk (Therms)
206	1.00	1.126
304	1.00	1.075
400	1.00	1.197
43	1.00	0.867
62	1.00	0.974
4	1.00	0.954
27	1.00	0.961
63	1.00	0.809
303	1.00	1.167
211	1.00	0.851

A Tentative Statement of the Requirements for Milk Production. It is evident that the data is not at hand to make it possible to give an accurate statement regarding the requirements for milk production. Before this is possible it will apparently be necessary to revise the figures in use as digestion coefficients since it is evident

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that the cow in milk falls considerably short of these figures which have been determined for animals on maintenance. Further, the production values for feed when used for milk production will have to be determined as it seems reasonably certain that the data along this line from experiments with fattening cattle are hardly applicable to dairy cows. The best that can be done at present is to express the requirements for milk production on the most accurate basis so far formulated which the author believes is Armsby's "Production Value" tables. In the suggested standard which is given below the energy requirement for 4.0% milk is that given by Armsby since it is borne out by our experimental results. The variation with the richness of the milk is based upon our data which has been given. The figure for digestible protein which in this case is albuminoids is based upon the standard of Armsby, the work of Savage and data obtained at this station. The experience of skillful feeders alone is sufficient evidence that a very high protein content is necessary for the sustained production of rich milk.

SUGGESTED STANDARD FOR COWS YIELDING MILK OF VARYING RICHNESS.

Per cent fat.	Digestible protein per pound milk.	Energy Therms per pound Milk
3.00	.050	.26
3.50	.052	.28
4.00	.055	.30
4.50	.058	.33
5.00	.062	.36
5.50	.066	.40
6.00	.070	.45
6.50	.075	.50

The following is suggested for herd feeding where it is not practical to take into account the richness of the milk of each individual.

	Digestible protein.	Energy therms
Holstein	.05	.26-.28
Shorthorn, Ayrshire, Brown Swiss	.055	.28-.30
Jerseys and Guernseys	.066	.40-.45

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SUMMARY

The object of the investigation was to secure data regarding the quirements for milk production by cows yielding milk of varying chness. Data is presented giving the feed consumed with chemical alyses, and of milk produced with analyses for eight cows for an tire year. The milk of these cows ranged from 3.4% to 6.09% t. Data of two other cows is included for shorter periods. These ws were all fed a ration of practically the same composition. The antity fed was regulated so as to maintain a uniform weight.

All cows were kept farrow. A maintenance trial was made for ven cows using the same ration as fed when in milk. A ten-day gestion trial was made for five of these when at maximum milk oduction and again when on maintenance. The digestible pro- in and the energy value of the rations received are calculated cording to Armsby's "Production Value" tables and also from e actual digestion coefficients. Comparison is made with Armsby's andard of energy required for milk of corresponding richness. he relation of the richness of the milk to the economy of fat pro- uction and to total energy in milk is also pointed out.

CONCLUSIONS.

The data bears out the results of others that more energy value required in the ration for rich milk than for milk lower in fat.

The maintenance requirements for the seven cows showed some ariation but was close to Armsby's standard for cows of the same eight.

The protein fed was in excess of that called for in the standard Armsby or Haecker but no attempt was made to determine the inimum requirement.

When the energy value of the ration, in excess of maintenance, as calculated by the use of "Production Value" tables it was found at the cow producing milk with 3.4% fat used .245 therms per ound while one with milk averaging 6.09% fat used .524 therms er pound.

When Haecker's maintenance requirement was used and the nount of "digestible nutrients" calculated by using Henry's tables was found that six cows used more than Haecker's standard and ur less. When actual maintenance was deducted every cow used utrients in excess of this standard. The deficiency increased with e richness of the milk. Four Jersey cows required from 23.1%

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to 55.2% more nutrients than called for by Haecker's standard. This standard is clearly too low for cows with rich milk.

According to average digestion coefficients the five cows should have digested 70.8% of the ration received during the digestion trial when in milk. The results showed only slight variation with individuals and an average of 65.57% digested. On maintenance the same cows should have digested 66.69% of the ration received according to average figures but the results were higher in every case and showed an average of 71.2%.

A calculation based upon the chemical analyses of the feed and the actual digestion coefficients showed the actual energy value used in the feed per pound of milk was lower than indicated by applying Armsby's "Production Value" tables directly to the ration received. The cow producing milk with 3.4% fat actually used only .235 therms per pound milk while for 6.09% fat the requirement was .442 therms.

The total energy required in the feed was slightly less per pound fat produced in the richer milk. However, after subtracting maintenance the energy per pound fat is consistently higher for the richer milk.

The cheaper production of fat in the richer milk is shown to be due to a smaller maintenance requirement per unit of fat on account of the smaller size of the animals producing the richer milk.

The energy value of the milk solids is greater in proportion to the energy value of the feed required with the milk lower in fat. This indicates the production of rich milk requires an increase in feed in excess of the increase in energy value of the milk.

The cow is able to utilize energy in her ration to better advantage than is indicated by Armsby's "Production Value" tables which are based upon experiments with mature fattening animals.

A tentative standard is given of energy value and protein for cows producing milk from 3% to 6.5% fat.

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The author wishes to acknowledge his indebtedness to O. E. Reed and G. C. White, assistants in this department while a portion of this data was being taken, and especially to T. E. Woodward who had complete charge of four of the experimental animals for a period of 8 months.

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RESEARCH BULLETIN NO. 8.

UNIVERSITY OF MISSOURI

COLLEGE OF AGRICULTURE

AGRICULTURAL EXPERIMENT STATION

THE KILLING OF PLANT TISSUE BY LOW TEMPERATURE

COLUMBIA, MISSOURI
December, 1913

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THE KILLING OF PLANT TISSUE BY LOW TEMPERATURE.

W. H. CHANDLER.

Summary.

1. The term sap density, as often used in this publication, refers, not to specific gravity, but to molar concentration. These sap densities have been determined by the freezing point method, making use of the fact that the molecular weight in grams of any non-electrolyte lowers the freezing point 1.86° C. The sap density is generally given in terms of depression, meaning the number of degrees Centigrade that the freezing point is lower than the freezing point of water.

2. By the eutectic point is meant the temperature at which the substance in solution crystallizes out. At that temperature there would be at the same time ice, crystals of the solute, and unfrozen solutions.

3. There are several forms of injury from cold, some of them purely mechanical, such as tearing of tissue due to tension developed at low temperature, or evaporation from the surface when the conducting tissue is frozen so as to prevent the movement of water to that tissue, and killing as a result of long continued exposure to low temperature. The term *freezing to death*, however, is applied here only to a very specific set of phenomena. With all plant tissues, when a certain temperature is reached very shortly after thawing, it will be found that the tissue has taken on a brown, water-soaked appearance, and evaporation from that tissue is much more rapid than from living tissue. These are characteristics of plant tissue frozen to death.

4. Results of many investigations have shown that during freezing (which may or may not result in freezing to death), ice forms in the tissue, generally not in the cells but in the intercellular spaces, the water moving out of the cells to form crystals in these spaces. The most commonly accepted theory is that killing from cold results from the withdrawal of water from the protoplasm. The amount of water loss necessary to result in death varies with the different plants and different tissues. (Pages 147-155).

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5. In the experiments described in this bulletin, the killing temperature of plant tissue that kills at relatively high temperature has been reduced whenever the sap density of the tissue has been increased. (Pages 155-187).

6. In addition to ripe apples and pears, and the leaves of *Agave Americana* observed by Müller-Thurgau and Molisch, leaves of lettuce kill at a slightly lower temperature if they are thawed slowly than if thawed rapidly. In case of all other tissues tested by this station or by others, including unripe apples and pears, there is no indication that the rate of thawing has anything to do with the amount of killing at a given temperature. (Pages 187-194).

7. Rapid wilting of tissue has not generally increased the resistance of plants to low temperature over that of unwilted tissue with a dry surface. However, tissue with a wet surface killed worse at a given temperature than did tissue with no moisture on the surface. (Pages 194-198).

8. Slow wilting or partial withholding of water through a long period increased the resistance of tissue to low temperature. (Pages 198-199).

9. In case of hardy winter buds and wood, a rapid decline in temperature greatly increased the severity of injury from a given low temperature. (Pages 199-218).

10. There seems to be no constant relation between the rate of growth of plant tissue and resistance to low temperature. Young leaves of fruit trees kill at a higher temperature than do old, mature leaves, while the young leaves of lettuce withstand a lower temperature than do the older leaves. (Pages 218-222).

11. Previous exposure to low temperature above that at which the tissue kills seems to increase the resistance of tissue to low temperature. (Pages 223-224).

12. The most important feature affecting the hardiness of plant tissue is maturity, that is, the condition of resistance that the plants reach during the winter dormant period. Maturity in the case of cambium may be intimately associated with the process of drying out. However, this can not be true at least of cortex of winter twigs. There is little difference between the moisture content of unfrozen cortex in seasons when it is very tender and seasons when it is hardy. The wood at the base of the trunk and at the crotches of all rapidly growing branches seems to reach a condition of maturity in early winter more slowly than does most other tissue. (Page 224).

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13. Of the tissue above ground during periods when the most complete maturity is reached, the most tender parts are the pith cells and the fruit buds. During periods of rapid growth there is little difference in hardiness of the different tissues. The tissue which is most tender at all seasons of the year is the root. There is much less difference, however, in the killing temperature of roots in summer and winter than between the killing temperature of twigs or other wood in summer and winter. (Pages 224-239).

14. Roots of the French crab used as stock seem to be more tender than roots which come from scions of an average variety of apple. (Pages 239-243).

15. Marianna plum roots are certainly more hardy than Myrobolan roots, and Mahaleb cherry roots seem slightly more hardy than Mazzard roots. (Pages 243-252).

16. That part of the root system nearest the surface and the largest, oldest roots are more resistant to cold than are small roots further from the surface. (Pages 233-252).

17. Pollen of the apple will withstand much lower temperatures than will any other tissue of the flower when in full bloom. (Page 253).

18. Scales of peach buds do not serve to protect them from low temperature. Buds frozen in the laboratory with the scales removed were slightly more resistant to low temperature than were buds with the scales not removed. (Pages 254-256).

19. The killing of wood of peach trees from freezing is one of the most important determining factors in peach growing. Little can be done to influence the amount of killing except to have the trees start into winter in proper condition of maturity. The weakest growing trees, however, do not generally reach this condition of maturity in the most satisfactory manner. Trees one or two years in the orchard, or old weak trees, are most liable to succumb to effects of low temperature. Pruning the trees severely following a winter when the wood has been killed, altho apparently in the best condition of maturity, seems to reduce the amount of killing. However, such pruning following winters when the wood has been killed on account of its not having reached the proper condition of maturity in the fall, generally due to the presence of wet weather following a drought the season before, is liable to result in greater loss than if no pruning were done. (Pages 256-266).

20. The hardiness of peach buds when in fully dormant condition seems to be greatly increased by continuous low temperature preceding the date at which the temperature goes low enough to kill. This capacity to withstand low temperatures seems likely to be due

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to the slow fall in temperature under such conditions rather than to hardiness developed as the result of exposure to low temperature. (Pages 266-269).

21. In the peach growing district of South Missouri and Arkansas, and probably other similar climates, the most important factor influencing the loss of peaches from low temperatures in winter is keeping the buds from starting into growth during warm periods in winter. In that section the best means of accomplishing this end is prolonging the growth of the trees in autumn, either by heavy pruning or by fertilizing with nitrogen the spring before. Some varieties of peaches have a much longer rest period than other varieties and therefore are started into growth more slowly by warm periods in winter. (Pages 269-283).

22. The killing temperature of peach blossoms when the tree is just coming into full bloom, under Missouri conditions, seems to vary from about 22° F. to 25 or 26° F. After the blossoms are old enough that they are probably pollinated, and from that time on until the peaches are as large as one-half inch in diameter, at least, they continue to become more tender until they will withstand but very few degrees below the freezing point, the seeds of young peaches killing at a higher temperature than other peach tissue. (Pages 283-286).

23. So far the investigations at this station indicate that early varieties of peaches are not started into growth more readily by warm periods in winter than are later varieties. Some of the very early varieties of the Chinese Cling group are the most slowly started into growth in early winter and bloom as late as any of the varieties. However, after blooming time these early peaches grow much more rapidly and are much more liable to be killed by a freeze after the fruit is set. (Pages 286-293).

24. Killing of wood of the apple is of considerable importance in some apple growing sections. Among the most common injuries are root killing, crown rot, crotch injury, sun scald, trunk killing and killing back of top and branches. (Pages 293-297).

25. Killing of apple buds from low temperatures is not common but has been observed. (Pages 297-298).

26. The blossoms and young fruit of the apple will not generally withstand as low temperature as will the blossoms or young fruit of equal age of the peach. (Pages 298-302).

27. While the killing of cherry and plum buds is less common than the killing of buds of the peach, such killing is often to be observed in some sections. The young fruit of the Wild Goose plum is among the most resistant to late frosts in spring. (Pages 302-303).

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STUDIES ON THE KILLING OF TISSUES OF HORTICULTURAL PLANTS BY LOW TEMPERATURE

The work to be reported in this paper was begun during the season of 1904-05. At that time the studies concerned only the effect of certain cultural methods on the hardiness of the fruit buds of the peach under climatic conditions that prevail in the southern half of Missouri. Later they were extended to include the possible effect that a large amount of potassium or other mineral elements in the soil might have on the ability of the peach fruit buds to withstand cold.

In taking up this problem it is necessary to distinguish between some of the various phases of injury from cold. Various writers have mentioned observations on frost cracks, that is, a formation of cracks in the wood of the tree during freezing weather. Caspary[1] showed that the formation of these cracks is due to a greater contraction of the tissue of the tree tangentially than radially. Later, Müller-Thurgau[2] made a very careful study of these cracks and confirmed Caspary's opinion, but showed that this great contraction tangentially is due rather largely to the shrinking of the cells of the medullary rays. These medullary rays extend in lines from the center out, and are made up of rather thin walled cells separated nowhere by very rigid tissue. These rays separate wider strips of rigid tissue extending in a wedge shape from the surface to the center with no lines of more pliable tissue crossing them. Then where the cells of the medullary rays contract on the passage of water into the intercellular spaces to form ice crystals, shrinking toward the center is limited to the rate of shrinking of the strong wedges of rigid tissue, while radially there is the shrinking of the rigid tissue and the more rapid shrinking of the medullary ray tissue.

There seems to be another type of injury[3] to the wood of trees, especially the small twigs, due apparently to the fact that during a long cold period much moisture will be lost from these twigs that can not be replaced because of the frozen condition of the conducting tissue. Thus death will result from the great loss of water from the twigs by evaporation. Killing of this kind seems to be worse in regions with prevailing strong winds and continuous low winter temperature. Thus Allen[4] finds a rather direct correlation between the

[1] Bot. Zeit. Vol. 13, pp. 449-62, 473-82, 489-500 (Bibl. No. 17); Bot. Zeit. Vol. 15, pp 329-35, 343-50, 361-71 (Bibl. No. 19).

[2] Landw. Jahrb. Vol. 15, p. 453, 1886 (Bibl. No. 78).

[3] A. Nelson, Wyo. Agr. Exp. Sta. Bul. 15, 1893. (Bibl. No. 81).

[4] Master's Thesis, Iowa Agr. Exp. Sta. (Bibl. No. 2).

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hardiness of the different varieties of apples and the rate at which water will be evaporated from their twigs.

Another form of injury, at least an injury that has been attributed to the effect of low temperature, results in the formation of dead areas on the bark of the tree trunk, especially near the top of the ground or in the crotches formed by the branches. Such injury has been studied by Goeppert[1], Sorauer[2], Grossenbacher[3] and others. Grossenbacher finds this injury greater on the side of the tree next to the prevailing wind, indicating that the great evaporation from the bark during a long period when it is frozen, and especially the tearing due to the bending of the tree when the bark is under high tension, may have something to do with this form of injury. This may not be a different form of injury from direct freezing to death which will be discussed later.

Sachs observed that the foliage of certain plants wilted following exposure to a temperature above the freezing point. He and also Müller-Thurgau[4] conclude that this wilting was not due directly to the effect of cold, but indirectly to the inability of the roots to take up moisture at so low a temperature to replace the evaporation from the leaves. According to Molisch[5], plants continuously exposed to a temperature too low for normal metabolism, but above the freezing point, will eventually die. Under these conditions, death ensues more slowly than where plants are killed by a sudden freeze. The plants gradually turn yellow and die, or dark colored dead spots are formed on the foliage.

Müller-Thurgau[6] limits the term freezing to death ("erfrieren") to the most common phenomena to which we have reference in speaking of killing from cold. It is death of the tissue following, directly, the lowering of the temperature below the freezing point, with the accompanying formation of ice crystals. When plant tissue is thus frozen to death in the case of growing plants, the foliage in practically all cases has a wilted or limp appearance. Pronounced color changes take place. Thus in most cases the green color due to the chlorophyll is lost, and the tissue takes on a brownish watery color. Plant cells containing coloring matter give up this coloring matter to the adjoining cells of the liquid in the intercellular spaces. Other

[1]Ueber die Wärmeentwickelung in dem Pflanzen, etc. Book, 1830. (Bibl. No. 44).
[2]Landw. Jahrb. Vol. 35, pp. 465-525. 1906. (Bibl. No. 105).
[3]N. Y. Geneva, Agr. Exp. Sta. Tech. Bul. 23, (Bibl. No. 50); N. Y. Geneva, Agr. Exp. Sta. Tech. Bul. 12, (Bibl. No. 51).
[4]Landw. Jahrb. Vol. 15, p. 453, 1886. (Bibl. No. 78).
[5]Untersuchung über das Erfrieren, etc. 1897, Book. (Bibl. No. 75).
[6]Landw. Jahrb. Vol. 15, p. 453, 1886. (Bibl. No. 78).

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color changes often take place, due to chemical changes when the coloring matter comes in contact with other substances from other cells. Death[1] can often be detected by these color changes. In the case of certain buds, and especially the stem and root tissue of hardy trees, the changes indicating this sort of death from cold can not be detected so soon after thawing, but are very characteristic, the tissue showing the watery, brownish appearance in a few hours after thawing. In all cases water is very rapidly lost from tissue killed in this way. Thus Goeppert[2] has shown that 24.25 grams of frozen canna leaves, which on thawing proved to be dead, lost when kept for six days in the open air near a warm stove, 21.25 grams, while the same weight of live canna leaves lost in the same length of time only 11.27 grams. When the killing temperature is barely reached, not all of the tissue is likely to be killed, but often there will be spots of dead tissue with live tissue adjoining. It has been observed by Sorauer and others, that the number of dead cells does not increase beyond those that are easily observed to be dead a short time after thawing. However, it requires longer for death to be plainly observed in the case of some tissues than others. As mentioned above, the tissue of the sap wood and cortex does not show plainly whether or not it is dead as soon as does the tissue of leaves and buds. Sorauer[3] found that epidermal tissue is slower in showing death after thawing than other tissues.

REVIEW OF THE LITERATURE ON FREEZING TO DEATH

In this paper the term freezing to death will be used as it was used by Müller-Thurgau, only when referring to the phenomena just described. While some early observers were of the opinion that plants have the ability to generate heat within their tissue and thus avoid severe freezing, the early Greek philosophers, observing the presence of ice within the plant tissue and not knowing of the cellular structure, were of the opinion that the injury was due to rending and mashing organs by the ice formation. Du Hamel and Buffon[4] were among the first to present a theory of the cause of death from cold based on a partial knowledge of cellular structure. They believed killing to be a rupturing of the cell walls due to the expansion accompanying ice formation.

[1]Molisch, Untersuchung über das, etc. 1897. Book. (Bibl. No. 75); Muller-Thurgau, Landw. Jahrb. Vol. 15, p. 453, 1886. (Bibl. No. 78).
[2]Ueber die Wärmeentwickelung, etc. Book, 1830. (Bibl. No. 44).
[3]Landw. Jahrb., Vol. 35, pp. 469-525, 1906. (Bibl. No. 105).
[4]Mem. d. l'Acad. Roy. Sci., Paris, 1737, pp. 273-298. (Bibl. No. 30).

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Goeppert[1] was among the first to make a careful study of killing from cold. He observed the formation of ice within the cells, and also in the intercellular spaces. Sachs[2] found that it was almost always in the latter.

Müller-Thurgau[3] in his very excellent studies found that when the ice crystals were found within the cells, it was due to very rapid freezing such as Goeppert used, and when the temperature was lowered very slowly, ice crystals were seldom found within the cell. Müller-Thurgau proved that ice formation within the tissue is necessary to freezing to death. It is well known that a liquid may often be supercooled several degrees below the freezing point before ice formation begins. He observed that this often occurs in cooling plant tissue in the laboratory, and when pieces of tissue (potato) were supercooled, if they could be warmed without ice formation they were not injured, while if ice formed they would kill at a higher temperature than that to which they were supercooled. Voigtlander measuring his temperatures with more delicate apparatus, has proved this perhaps more conclusively. When he supercooled tissue to four or five degrees centigrade below the point at which it would kill when ice formed, if the temperature could be raised to above the freezing point without ice formation, killing never occurred. It was held by many scientists, at the time of Müller-Thurgau's first work, as well as by a large majority of practical observers, that death was due not directly to low temperature, but to rapid thawing. Goeppert was of the opinion, however, from his studies, that the killing was a direct result of freezing and that death actually occurred before the thawing began. Sachs, from some experiments with plants immersed in cold water to thaw, after freezing, held that the amount of killing of the plants at any given temperature was determined by the rate of thawing. Müller-Thurgau showed that the method used by Sachs of thawing plants in cold water was not a method of slow thawing, but rather a very rapid thawing since a layer of ice crystals would form on the outside of the tissue, giving off heat that would thaw the tissue very rapidly. In fact the tissue thawed in cold water much more rapidly than in the air at room temperature.

Müller-Thurgau using a large number of plants, thawing them from the same temperature, some rapidly and some more slowly,

[1]Ueber die Wärmeentwickelung, etc. Book. 1830. (Bibl. No. 44).

[2]Ber. u. d. Ver. d. Kon. Sachs. Gesell. d. Wiss. zu Leipzig, 1860, Vol. 12, pp. 1-50. (Bibl. No. 94).

[3]Landw. Jahrb. Vol. 15, p. 453, 1886. (Bibl. No. 78).

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as never able to detect any difference in the amount of killing when awed rapidly or slowly, except in the case of the fruit of the apple nd pear. Molisch[1], following the work of Müller-Thurgau, tried so slow and rapid thawing from the same temperature with a very rge number of plants, and found that in nearly all cases the rate of awing had nothing to do with the killing. In the case of the fruit the apple and pear, and the leaves of Agave Americana, the slow awing gave less injury, but even with these, a slightly lower temerature than that at which they kill with rapid thawing, would kill em, regardless of the rate of thawing. Müller-Thurgau observed refully the freezing of tissue under the microscope and found that e was very seldom formed within the cell, but usually ice crystals rmed outside the cell in the intercellular spaces and continued to crease in length as the temperature went lower, the water passing om the cell into the intercellular spaces increasing the length of e crystals.

By placing plant tissue frozen to various temperatures in 100 . of water carefully insulated, and noting the temperatures to which e water was lowered, excluding the losses of heat for warming up e plant tissue, correcting for the heat of the beaker, etc., making e of the fact that eighty gram calories are required to melt one am of ice, Müller-Thurgau[2] was able to determine, apparently ith some accuracy, the percentage of the plant water that is frozen to ice at various temperatures. Only plant tissue with a deterined moisture content was used. With the apple he gives the folwing percentages of water frozen out at given temperatures: at .5°, 63.8 per cent of the water was frozen; at -13°, 74.4 per cent of e water was frozen; at -15.2°, 79.2 per cent of the water was frozen e also attempted to measure the percentage of the water frozen out woody tissue by means of frost cracks. His method was to freeze section of a young tree trunk until a frost crack of a certain width as formed. On thawing of the tissue this crack would close. His xt step was to dry the section of tree trunk until a frost crack of e same width was formed. He assumed that the percentage of ater loss necessary to form this crack is equal to the percentage ken from the cell during freezing sufficient to form an ice crack of e same width.

Molisch studied with great care, under the microscope, the eezing of various plant tissues, observing the same phenomena de-

[1]Untersuchung über das, etc. 1897, book. (Bibl. No. 75).
[2]Landw. Jahrb. Vol. 9, p. 453, 1886. (Bibl. No. 78).

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scribed by Müller-Thurgau with reference to the freezing of the cell water in the intercellular spaces, rather than within the cells. Both Müller-Thurgau and Molisch hold the view that freezing to death results from the rapid withdrawal of water from the cells to form these ice crystals in the intercellular spaces.

Matruchot and Molliard[1] observed that water is extruded from the nuclei of plants that have been frozen, dried or subjected to the action of solutions of high osmotic concentration.

Gorke has recently offered an interesting theory as to the cause of death by freezing. He found that when the plant sap is frozen, certain proteids may be precipitated out and apparently those plants that are most easily killed by freezing have their proteids precipitated out at the highest temperature. Thus begonia, which is very easily killed, had its proteids precipitated at -3° while sap from pine needles required a temperature of -40° to precipitate any proteids. Gorke[2] assumes then that killing from cold may be due to the precipitation of the proteids, and accounts for this precipitation by the greater concentration of the salts in the sap as water is removed to form ice crystals. It is well known that certain proteids can be precipitated out by increasing the concentration of salts, especially zinc sulphate and ammonium sulphate. Gorke made up solutions of albumen with zinc sulphate and found that after freezing to -20° there was a large precipitation of proteids.

Lidforss[3] working with the wintergreen plants of South Sweden, has found that with most of them at least during cold weather, the starch is almost entirely changed to sugar, though on the return of warm weather starch may be again deposited in the cell. He assumes that this sugar is formed during cold weather as a means of protecting the plant against freezing by lowering the freezing point of its sap. He was able also to increase the resistance to low temperature of the leaves of wintergreen plants and the roots of *Zea Mays* by keeping them for a time immersed in 5 to 10 per cent sugar solutions.

Schaffnit[4], following the work of Gorke, found that the proteids of rye grown in the open at low temperatures are not readily precipitated by freezing, while the same temperature will readily precipitate proteids from sap of rye grown in the greenhouse at much

[1]Compt. Rend. Acad. Sci. Paris, Vol. 132 (1901) pp. 495-8. (Bibl. No. 71a).

[2]Land. Versuchs. Vol. 65, p. 149, 1906. (Bibl. No. 47).

[3]Lunds Universitets Arssk., Vol. 2, No. 13, 1907 (Bibl. No. 62); Bot. Centlb., Vol. 68 No. 2, p. 33. (Bibl. No. 63).

[4]Mitt. Kaiser Wilhelm Inst. Landw. Bromberg, Vol. 3, No. 2, pp. 93-115, (Bibl. No. 96); Zeits. f. Allg. Phys., Vol. 12, pp. 323-36. (Bibl. No. 99).

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higher temperatures. He also found that he could prevent the precipitation of proteids from this sap of greenhouse rye by adding to it small quantities of sugar. He concludes then that the formation of sugar in wintergreen plants described by Lidforss may be the means of protecting the plants against precipitation of proteids. However, Schaffnit concludes that precipitation of proteids is the only way by which loss of water during freezing kills plant tissue.

Maximow[1] has recently published three very interesting papers covering work in freezing sections of plants, mainly red cabbage and *Tradescantia discolor*. Thin sections of tissue from the upper side of the leaves were frozen in solutions of various strengths of both organic and inorganic substances after they had stood for varying lengths of time in the solutions. He found remarkable protection to be exerted by both organic and inorganic substances whenever their eutectic point (the temperature at which they crystallize out, giving mixtures of solute crystals with the ice crystals) does not lie too near the freezing point and whenever the substance is not excessively toxic. He used strengths varying from $\frac{N}{16}$ to 2N of glucose and glycerine, and $\frac{N}{8}$ to 2N of methyl and ethyl alcohol, and mannite. Of inorganic substances he used solutions with strengths of 0.1N to 2N of sodium chloride, potassium chloride, calcium chloride, sodium nitrate, potassium nitrate, calcium nitrate, sodium acetate, potassium acetate, calcium acetate, sodium lactate, potassium lactate, sodium oxalate, and potassium oxalate, also magnesium nitrate, magnesium chloride, ammonium nitrate, ammonium chloride, sodium sulphate, and potassium sulphate.

According to Maximow, mannite, sodium sulphate, potassium sulphate. potassium nitrate, and sodium oxalate show little protection because of their high eutectic point; and magnesium chloride, magnesium nitrate, and ammonium chloride because of their toxicity, while calcium chloride and calcium nitrate show reduced protection because of their toxicity. All other solutions, however, showed great protection that was very uniform for the same osmotic concentration. Sometimes a temperature as low as -32° did not kill all of the cells of the red cabbage. Probably the most interesting result of Maximow's work was the observation that when the sections were immersed in these solutions and immediately frozen, as much protection was exerted as when they had been permitted to remain in the solutions for twenty-four hours or longer. The tender Tradescantia cells immersed in expressed sap of the red cabbage and im-

[1]Ber. der Deutsch. Bot. Gesell., Vol. 30, pp. 52-65, 293-305, 504-16, (Bibl. No. 73).

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mediately frozen, would actually withstand more cold than the hardier red cabbage when frozen in winter. Maximow concludes that the part of the cell which is injured when exposed to low temperature is the plasma membrane, and that as long as a film of water was kept in contact with this membrane, death was not likely to occur His theory then would not be greatly different from that of Müller-Thurgau and Molisch, that withdrawal of water kills, except that in Maximow's opinion killing following the withdrawal of water seems to be limited to the plasma membrane.

If Maximow's work is verified by further experimenting, using other plants, it is certainly a very interesting contribution toward determining just what freezing to death of plant tissue is.

Mez[1] studied the effect of supercooling upon plant tissue. He finds that where ice formation begins at once on reaching the freezing point, the killing is not so great as where there is supercooling when large masses of ice are formed rapidly after crystallization begins. By use of the thermo-couple he studied the fall of temperature in the plant, using stems of *Impatiens* to determine the eutectic points of the sap solutes. At each of these points there will be a halting in the temperature fall due to the heat given off on crystallization. From this work he concludes that when a temperature of -6° is reached, all solutes will crystallize out. He thinks this should disprove the theory of Müller-Thurgau and Molisch, since there should be complete loss of water at this temperature and the plant should never survive a lower temperature if loss of water from the cell is the cause of death. He holds that the heat liberated by the crystallizing of the solutes and the formation of ice, will after the cells are insulated by the ice mass, aid in keeping the temperature of the cell above that of the surroundings. He holds, therefore, that each plant has its specific minimum point at which death occurs due to the direct effect of the cold, and that if supercooling takes place, large amounts of heat are lost before the cells are insulated by the ice mass and therefore this specific minimum will be more quickly reached. The work of Müller-Thurgau[2] and of Voigtlander[3], (a pupil of Mez) where plants supercooled to below the killing temperature remained alive if ice did not form, certainly refutes the theory of Mez. If further evidence were needed, the protective action of organic and inorganic substances shown in Maximow's work certainly proves the fallacy of Mez's conclusion. Even his conclusion that the sap solute

[1]Flora, Vol. 94, p. 89, 1905. (Bibl. No 74).
[2]Landw. Jahrb. Vol. 15, p. 453, 1886. (Bibl. No. 78).
[3]Beitr. z. Biol. der Pfl. Cohn. Vol. 9. 1909. pp. 359-414. (Bibl. No. 110).

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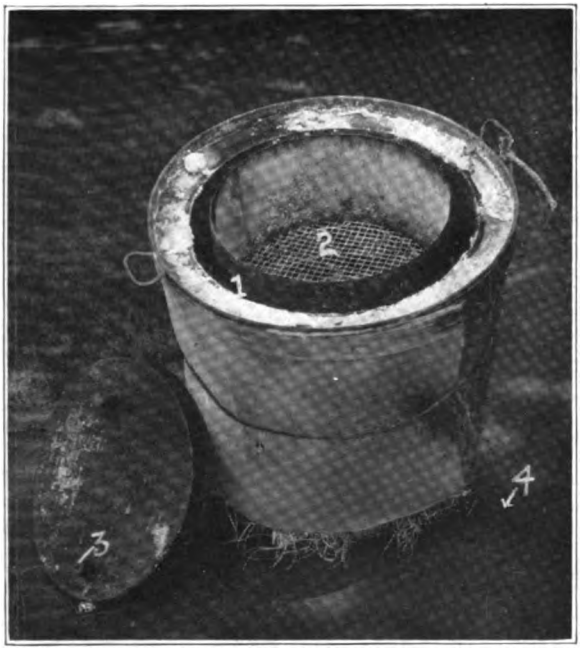

FIGURE I.—FREEZING APPARATUS FOR PLANTS THAT KILLED AT A TEMPERATURE NOT LOWER THAN −12° TO −15° C.

1. Space in which salt and ice mixture was placed;
2. Chamber in which plants were frozen;
3. Lid which covered freezing chamber;
4. Wire leading to small electric fan beneath hardware cloth bottom on which plants were frozen. (See page 156.)

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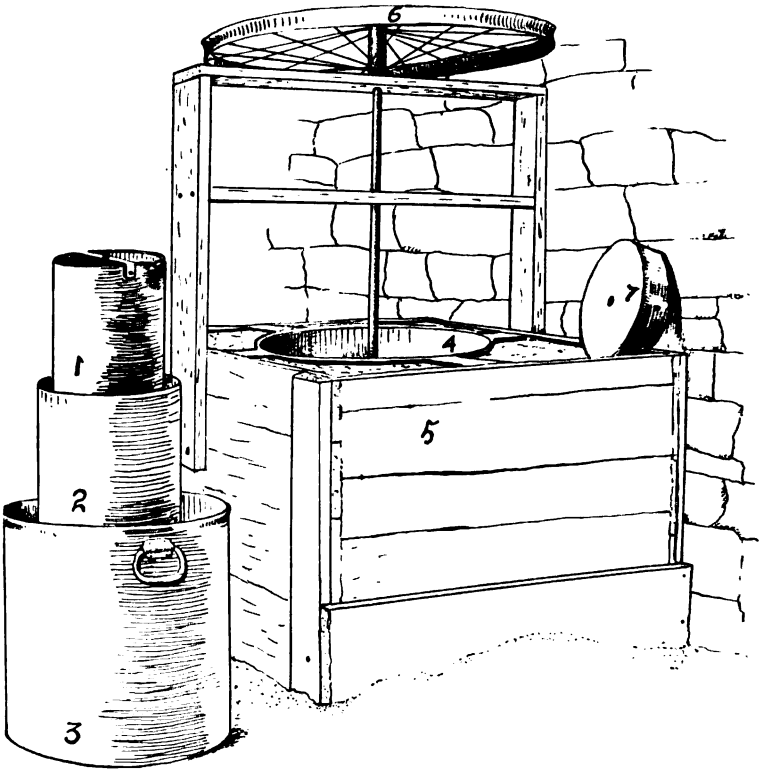

FIGURE II.—APPARATUS FOR FREEZING TISSUE THAT REQUIRED LOWER THAN −12° TO −15° C. TO KILL.

1. Galvanized iron cylinder to the bottom of which the twigs containing buds were fastened; 2 and 3. Galvanized iron cylinders placed one within the other with space between their walls for freezing mixture; 4. Cylinder to receive 3, allowing space for freezing mixture around 3; 5. Insulating box filled with sawdust; 6. Wheel operated by electric motor and belt in order to keep (1) turning continuously while freezing; 7. Lid for (2). (See page 157.)

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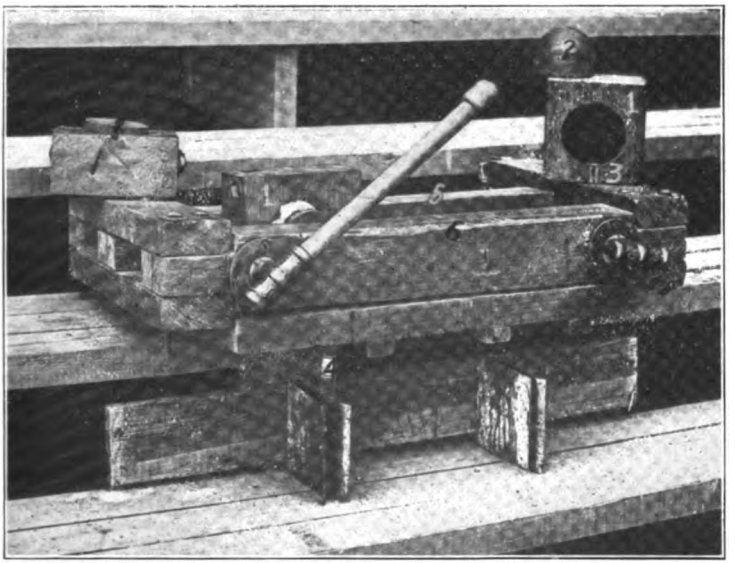

FIGURE III.—APPARATUS FOR EXPRESSING PLANT JUICE.

The two blocks between which the plant tissues were pressed are shown at (1) and (2). The juice escaped through a hole (3) in block (1). The plant tissue was placed between the blocks (1) and (2), which in turn were placed between the pieces of movable 4″x4″ pieces which are drawn together by bench-screws. **(See page 158.)**

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must all be crystallized out at -6° C. can not be true since sugars would remain in solution at lower temperatures than that. Further, when we have evaporated the cortex sap of peach twigs in winter condition to one-sixth or one-eighth of its volume without permitting the temperature to go above 50° C. ice would not form when the temperature was lowered to -22° C. though many of the buds would be killed at that temperature. Mez seems also to ignore the force of imbibition which would tend to hold the water in the protoplasm even after the sap solute may be crystallized out. The fact, however, that when great supercooling takes place, plants are more liable to be killed, is of interest and is, of course, associated with the fact which will be discussed later that rapid cooling is more injurious to plant tissue than is slow cooling.

EFFECT OF SAP DENSITY ON KILLING TEMPERATURE.

If the theory of Müller-Thurgau and Molisch be true (even as it is modified by Maximow) it would seem that some plants might be hardy because the plasma membrane has the property of withstanding great loss of water. Some might be relatively hardy because of a property by which sufficient water to protect the plasma membrane at low temperatures is prevented from freezing, and some might be relatively hardy on account of the presence of both conditions. It is generally considered that after the effect of the sap solute in holding water unfrozen is exhausted, there is still left the force of imbibition. The relative importance of these two forces, however, is not determined. Disregarding the force of imbibition (which, however, may be the more important), it would appear to be true that if the sap density (by sap density is meant not specific gravity but molar concentration of the sap; that is, the number of gram molecules of the sap solute in one thousand grams of water) were doubled, then at any given temperature below the freezing point, but above the eutectic point of the solute, twice as much water would be held unfrozen to protect the protoplasm.

With this idea in mind, experiments were started in September, 1908, to determine whether or not an increase in the sap density would lower the killing temperature.

Seedlings of corn, cowpeas, garden peas, tomatoes, squash, cabbage and lettuce were grown in sand and watered with varying strengths of potassium chloride and ammonium chloride at first—later magnesium chloride, sodium chloride and sodium nitrate were also used—while check plants were grown under similar conditions

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except that they were watered only with distilled water. The plants were permitted to grow only as long as they would make good growth in the sand, probably about the time the food supply of the seed was becoming exhausted. Some of the plants of each set were then frozen while others had the sap expressed for osmotic strength (freezing point) determination by the use of a Beckmann apparatus. As a measure of the osmotic strength the term depression will be used in the tables, meaning of course the number of degrees centigrade below zero at which, with no supercooling, ice formation begins in the sap.

Method of Freezing. At first an effort was made to grow the plants in the greenhouse and expose them to outside temperatures to determine the killing temperature. However, this was soon found to be unsatisfactory and the plants were frozen in a chamber surrounded by a freezing mixture made of salt and ice. It is evident that the temperature throughout such a chamber would not be uniform so long as it were falling and great care was necessary to secure as uniform a temperature as possible. The apparatus shown in Fig. 1 was used. In the lower part was an electric fan; the upper part was the chamber in which the plants were frozen. An effort was made to keep the temperature uniform within this chamber by the operation of the electric fan just beneath the hardware cloth shelf on which the plants were frozen. Careful tests showed that the temperature throughout this chamber was always uniform on the same level though sometimes it would vary slightly in different levels. Fearing, however, that this would not always be true, the plants on freezing were always placed, not only on the same level, but at the same distance from the galvanized iron wall of the chamber. In this way it was practically impossible that plants in the freezer would not all be exposed to the same temperature, and consistent results were secured.

In addition to being sure that the plants were at a uniform temperature, it was necessary to control very carefully the rate of fall of the temperature, since rapid falling of temperature very greatly increases the killing. For this reason it was practically impossible to secure results that would be sufficiently accurate so that one freezing could be compared with another, except where differences were wide; that is, the plants to be compared must be frozen at the same time. However, when the differences are large it is possible to make a fairly accurate estimate of the relative hardiness of the plants frozen at different times, if great care is taken to duplicate as nearly as possible, the rates of temperature fall. It was found possible to

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ower the temperature of all the plants together to a point that would robably kill the most tender, and after removing these to lower it urther. The rate of fall would thus be the same for all down to the emperature at which the most tender were removed.

The thermometer used in the earlier years of freezing was a entane thermometer graduated to one-half degrees. The zero oint was far enough above the bulb so that when the thermometer as inserted through a cork at the top of the jacket of the freezing hamber, the bulb would be on the same level with the plants. Later, pecial mercury thermometers graduated to low temperature were sed. These were standardized by the makers. However, new hermometers were checked by those used with previous work, and lso, checked from time to time, with standard thermometers of the Columbia Branch of the United States Weather Bureau. No effort was nade to read the thermometers to closer than one-half degrees. Plants n being removed from the freezer were always examined to see if the issue were frozen stiff. In freezing buds and woody tissue that illed at a temperature lower than -15°, the apparatus shown in igure 2 was used. The twigs or pieces of tissue and the thermometer were fastened to the inner cylinder which was filled with cotton. This was set in a cylinder enough larger to leave a surrounding space of about three-fourths of an inch. The second cylinder was about ix inches taller than the inner one, and was set with a one and one-alf inch space between them. There was about two inches of space etween the walls of this third cylinder and those of the one in which his was placed. The fourth cylinder was well insulated by being acked in dry saw dust. Ice and salt were first packed loosely and hen firmly in the space between the fourth and third cylinder. In his way the temperature of the twigs could be lowered generally to 17° C. When it was necessary to secure a lower temperature, the pace between the third and second cylinders was packed loosely and ater firmly with salt and ice. In this way the temperature of the ir surrounding the inner cylinder could be gradually lowered at the ate of two to three degrees an hour after the freezing point was eached. By packing salt and ice to the top of the second cylinder he temperature from top to bottom of the inner cylinder would ary but little. However, the freezing tissue to be compared and the hermometer bulb were always kept the same distance from the bot-om of the cylinder. Since these cylinders were of galvanized iron nd would conduct heat rapidly, it would seem probable that the tem-erature around the central cylinder would not vary. However, earing that there might be some such variation in temperature, the

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central cylinder with the twigs or woody tissue and the thermome
on its surface was kept slowly revolving by means of a small elect
motor. During the freezing, this was stopped only for thermome
readings which were taken generally every fifteen minutes.

Method of Determining Freezing Point of Sap. The densi
of sap was determined by means of a Beckmann Freezing Point App
ratus. The tissue was ground in an ordinary food grinder, using t
knife that grinds the finest, and the sap was expressed with the app
ratus shown in Figure 3. The large block with the hole in the cen
is of sugar maple which will not split readily. The smaller blo
is of the same material and is made to fit in the depression in the lar
block leaving about one-eighth of an inch surrounding space. T
ground tissue was wrapped in a clean piece of eight or ten ounce du
and put in the depression in the large block, the small block p
against it and the two pressed together between pieces of 4x4 lumb
drawn together by a pair of bench screws as shown in the figu
The sap could be expressed very quickly. With succulent plan
loss by evaporation in all cases was negligible. In the case of leav
of peaches it required a considerable length of time for the sap
exude.

In all cases before plants with different treatments were froz
or before they were ground for expressing sap, they were, after bei
pulled, kept with the roots in a glass of water until they becam
apparently turgid, since wilting sometimes seems to reduce sligh
the killing temperature, and would appreciably affect the sap densi
determinations.

EXPERIMENTS WITH SEEDLINGS OF ZEA MAYS

A large number of corn seedlings were grown and frozen. T
following table gives the date of freezing, the solution with whi
the material was watered, the temperature to which the plants we
subjected, the percentage killed and percentage partly killed, and t
freezing point of the sap. The freezing point is given as depressio
meaning the number of degrees below zero, centigrade, at which i
begins to form in the sap, assuming no supercooling.

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TABLE 1. SHOWING EFFECT OF WATERING WITH MINERAL SOLUTIONS ON SAP DENSITY AND HARDINESS IN ZEA MAYS PLANTS.

Watered With	Date	Temperature	Number Plants	Percentage Killed	Percentage Killed and Partly Killed	Depression
Potassium Chloride (.0804 N).....	Dec. 12,'08	–3	6	33.3	83.3	1.10
Potassium Chloride (.0402 N).....	Dec. 12,'08	–3	6	16.6	100	1.06
Ammonium Chloride (.0804 N).....	Dec. 12,'08	–3	6	33.3	100	.94
Ammonium Chloride (.0402 N).....	Dec. 12,'08	–3	6	50	100	1.19
Potassium Chloride (.0804 N).....	Dec. 15,'08	–3	6	33.3	33.3	1.265
Potassium Chloride (.0402 N).....	Dec. 15,'08	–3	5	40	60	.94
Ammonium Chloride (.0804 N).....	Dec. 15,'08	–3	4	0	0	1.16
Ammonium Chloride (.0402 N).....	Dec. 15,'08	–3	6	100	100	.875
Potassium Chloride (.0804 N).....	Dec. 16,'08	–3.25	7	42.8	71.7	1.26
Potassium Chloride (.0402 N).....	Dec. 16,'08	–3.25	7	14.3	57.1	.94
Ammonium Chloride (.0804 N).....	Dec. 16,'08	–3.25	6	50	100	1.16
Ammonium Chloride (.0402 N).....	Dec. 16,'08	–3.25	9	33.3	88.9	.875
Potassium Chloride (.0804 N).....	Jan. 9,'09	–7	13	46.2	61.5	1.315
Potassium Chloride (.0402 N).....	Jan. 9,'09	–7	11	63.6	81.8	1.195
Ammonium Chloride (.0804 N).....	Jan. 9,'09	–7	9	77.8	100	1.005
Ammonium Chloride (.0402 N).....	Jan. 9,'09	–7	12	66.6	100	.935
Potassium Chloride (.0804 N).....	Jan. 9,'09	–6.5	11	27.3	54.5	1.315
Potassium Chloride (.0402 N).....	Jan. 9,'09	–6.5	13	61.5	84.6	1.195
Ammonium Chloride (.0804 N).....	Jan. 9,'09	–6.5	10	80	90	1.005
Ammonium Chloride (.0402 N).....	Jan. 9,'09	–6.5	14	50	85.7	.935
Potassium Chloride (.0804 N).....	Jan. 9,'09	–7.5	13	61.5	76.9	1.315
Potassium Chloride (.0402 N).....	Jan. 9,'09	–7.5	12	75	83.3	1.195
Ammonium Chloride (.0804 N).....	Jan. 9,'09	–7.5	8	87.5	100	1.005
Ammonium Chloride (.0402 N).....	Jan. 9,'09	–7.5	12	91.6	91.6	.935
Potassium Chloride (.0804 N).....	Jan. 19,'09	–3	12	16.6	50	1.315

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Watered With	Date	Temperature	Number Plants	Percentage Killed	Percentage Killed and Partly Killed	Depression
Potassium Chloride (.0402 N).....	Jan. 19,'09	–3	16	18.8	43.8	1.195
Ammonium Chloride (.0804 N).....	Jan. 19,'09	–3	17	11.8	41.3	1.005
Ammonium Chloride (.0402 N).....	Jan. 19,'09	–3	11	27.3	54.5	.935
Potassium Chloride (.0804 N).....	Jan. 19,'09	–6.5	20	25	40	1.315
Potassium Chloride (.0402 N).....	Jan. 19,'09	–6.5	16	25	37.5	1.195
Ammonium Chloride (.0804 N).....	Jan. 19,'09	–6.5	11	66.6	66.6	1.005
Ammonium Chloride (.0402 N).....	Jan. 19,'09	–6.5	18	11.1	16.7	.935
Potassium Chloride (.0804 N).....	Jan. 19,'09	–6	13	7.7	15.4	1.315
Potassium Chloride (.0402 N).....	Jan. 19,'09	–6	12	0	0	1.195
Ammonium Chloride (.0804 N).....	Jan. 19,'09	–6	11	0	0	1.005
Ammonium Chloride (.0402 N).....	Jan. 19,'09	–6	12	0	0	.935
Potassium Chloride (.0804 N).....	Jan. 21,'09	–4.5	21	61.9	66.6	.93
Potassium Chloride (.0402 N).....	Jan. 21,'09	–4.5	19	68.4	68.4	.92
Ammonium Chloride (.0804 N).....	Jan. 21,'09	–4.5	15	86.7	93	.995
Ammonium Chloride (.0402 N).....	Jan. 21,'09	–4.5	19	57.9	63.2	.88
Potassium Chloride (.0804 N).....	Jan. 21,'09	–4	16	0	12.5	.93
Potassium Chloride (.0402 N).....	Jan. 21,'09	–4	17	17.6	23.5	.92
Ammonium Chloride (.0804 N).....	Jan. 21,'09	–4	17	17.6	23.5	.995
Ammonium Chloride (.0402 N).....	Jan. 21,'09	–4	16	37.5	50	.88
Potassium Chloride (.0804 N).....	Feb. 2,'09	–5	10	0	50	1.49
Potassium Chloride (.0402 N).....	Feb. 2,'09	–5	9	11.1	66.7	1.143
Ammonium Chloride (.0804 N).....	Feb. 2,'09	–5	11	0	54.5	1.17
Ammonium Chloride (.0402 N).....	Feb. 2,'09	–5	13	30.8	38.5	1.325
Potassium Chloride (.0804N), average.....				29.6	51.3	1.238
Potassium Chloride (.0402N), average.....				34.3	58.9	1.091
Ammonium Chloride (.0804N), average.....				42.6	64.1	1.037
Ammonium Chloride (.0402N), average.....				46.3	65.8	.969

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It will be seen from these tables that by taking an average of a large number of these freezings, the percentage of killing is uniformly lower when the depression is increased.

Shaded Zea Mays seedlings were watered with .0804 N potassium chloride, and others with water with results as follows:

TABLE 2. SHOWING EFFECT OF WATERING WITH MINERAL SOLUTIONS ON SAP DENSITY AND HARDINESS OF SHADED ZEA MAYS PLANTS.

Watered With	Date	Temperature	Number of Plants	Percentage Killed	Percentage Killed and Partly Killed	Depression
Potassium Chloride (.0804 N)	Feb. 2,'09	−3	18	5.6	5.6	.93
Water	Feb. 2,'09	−3	17	17.6	17.6	.835
Potassium Chloride (.0804 N)	Feb. 2,'09	−5	17	41.2	58.8	.93
Water	Feb. 2,'09	−5	14	78.6	78.6	.835
Potassium Chloride (.0804 N)	Feb. 2,'09	−5	14	64.3	85.7	.93
Water	Feb. 2,'09	−5	15	80	80	.833
Potassium Chloride (.0804 N)	Feb. 12,'09	−5	8	12.5	50	1.22
Water	Feb. 12,'09	−5	7	57.1	71.4	.65
Potassium Chloride (.0804 N)	Feb. 12,'09	−4.5	8	50	75	1.22
Water	Feb. 12,'09	−4.5	9	100	100	.653
Potassium Chloride (.0804N), average				34.7	55.0	1.046
Water, average				66.7	69.5	.761

The following table gives results of freezing cowpea seedlings that had been watered with solutions containing 6.03% normal potassium chloride, sodium chloride, magnesium chloride, ammonium chloride, sodium nitrate, and distilled water.

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TABLE 3. EFFECT OF WATERING WITH MINERAL SOLUTIONS ON SAP DENSITY AND HARDINESS OF COWPEAS.

Watered With	Date	Temperature	Number Leaves	Percentage Killed	Percentage Killed and Partly Killed	Depression
Potassium Chloride....	June 29,'11	-3.5	35	45.71	57.14	1.05
Sodium Chloride.....	June 29,'11	-3.5	35	28.57	48.57	1.065
Magnesium Chloride..	June 29,'11	-3.5	33	69.69	100.00	.975
Ammonium Chloride...	June 29,'11	-3.5	33	3.03	15.15	1.035
Sodium Nitrate.....	June 29,'11	-3.5	33	9.09	33.33	1.05
Distilled Water.....	June 29,'11	-3.5	40	77.50	77.50	.825
Potassium Chloride....	June 29,'11	-3.5	30	56.66	70.00	1.05
Sodium Chloride.....	June 29,'11	-3.5	36	30.55	38.88	1.065
Magnesium Chloride...	June 29,'11	-3.5	36	66.66	77.77	.975
Ammonium Chloride...	June 29,'11	-3.5	29	58.62	75.86	1.036
Sodium Nitrate.....	June 29,'11	-3.5	24	70.83	79.16	1.05
Distilled Water.....	June 29,'11	-3.5	36	91.66	97.22	.825
Potassium Chloride....	July 18,'11	-3	21	33.33	42.85	1.13
Sodium Chloride.....	July 18,'11	-3	22	36.36	54.54	1.17
Magnesium Chloride...	July 18,'11	-3	30	85.33	100.00	1.00
Ammonium Chloride...	July 18,'11	-3	12	83.33	91.66	1.155
Sodium Nitrate.....	July 18,'11	-3	12	0.0	41.66	1.23
Distilled Water.....	July 18,'11	-3	30	90.00	100.00	.78
Potassium Chloride....	July 19,'11	-2.75	12	100.00	100.00	1.13
Sodium Chloride.....	July 19,'11	-2.75	12	66.66	66.66	1.17
Magnesium Chloride...	July 19,'11	-2.75	29	93.10	100.00	1.00
Ammonium Chloride...	July 19,'11	-2.75	12	83.33	100.00	1.155
Sodium Nitrate.....	July 19,'11	-2.75	12	0.0	58.33	1.23
Distilled Water.....	July 19,'11	-2.75	30	76.66	83.33	.78
Potassium Chloride....	July 20,'11	-2.75	12	16.66	58.33	1.13
Sodium Chloride.....	July 20,'11	-2.75	12	8.33	33.33	1.17
Magnesium Chloride...	July 20,'11	-2.75	12	58.33	75.00	1.00
Ammonium Chloride...	July 20,'11	-2.75	12	16.66	50.00	1.155
Sodium Nitrate.....	July 20,'11	-2.75	12	58.33	83.33	1.23
Distilled Water.....	July 20,'11	-2.75	30	80.00	93.33	.78
Potassium Chloride....	July 21,'11	-2.75	12	8.33	16.66	1.13
Sodium Chloride.....	July 21,'11	-2.75	12	0.00	8.33	1.17
Magnesium Chloride...	July 21,'11	-2.75	13	15.39	30.77	1.00
Ammonium Chloride...	July 21,'11	-2.75	12	8.33	16.66	1.155
Sodium Nitrate.....	July 21,'11	-2.75	12	16.66	16.66	1.23
Distilled Water.....	July 21,'11	-2.75	30	86.66	93.33	.78
Potassium Chloride, average.....				43.45	57.49	1.10
Sodium Chloride, average.....				28.41	41.72	1.135
Magnesium Chloride, average.....				64.75	80.59	.991
Ammonium Chloride, average.....				33.05	58.22	1.115
Sodium Nitrate, average.....				25.82	52.08	1.17
Distilled Water, average.....				83.73	90.78	.795

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It may be said, however, that the solutions all reduced the growth of the plants, as indicated by the following weights:

Average weight of plants watered with Potassium Chloride.....2.27 grams
Average weight of plants watered with Sodium Chloride.....1.89 grams
Average weight of plants watered with Magnesium Chloride.....2.33 grams
Average weight of plants watered with Ammonium Chloride.....1.78 grams
Average weight of plants watered with Sodium Nitrate.....1.68 grams
Average weight of plants watered with Distilled Water.....2.63 grams

The percentage of killing will thus be seen to be as much in proportion to growth as in inverse proportion to depression.

Corn seedlings were also grown where water was withheld, being watered only when it was necessary to keep them from dying. The following table gives the results:

TABLE 4. SHOWING EFFECT OF WITHHOLDING WATER ON SAP DENSITY AND HARDINESS OF ZEA MAYS.

Treatment	Date	Temperature	Number Plants	Percentage Killed	Percentage Killed and Partly Killed	Depression
Well watered.....	Feb. 2,'09	−4	13	84.6	92.3	.785
Water withheld.....	Feb. 2,'09	−4	10	0.0	40.	1.07
Well watered.....	Feb. 2,'09	−5	13	61.5	74.6	.835
Water withheld.....	Feb. 2,'09	−5	5	20.0	80.0	1.07
Well watered.....	Feb. 2,'09	−5.5	12	66.7	100.0	.835
Water withheld.....	Feb. 2,'09	−5.5	6	16.7	50.0	1.07
Well watered.....	Feb. 12,'09	−4.5	10	60.0	60.0	.71
Water withheld.....	Feb. 12,'09	−4.5	9	44.5	44.5	1.085
Well watered, average.....				68.19	81.72	.791
Water partially withheld, average.....				20.27	53.60	1.074

It will be seen again that withholding water increased the sap density (depression) and lowered the killing temperature. It also reduced the rate of growth and probably the size of the cells, so we can not conclude with certainty that the greater hardiness is due only to the greater sap density.

Tomatoes were grown in the same way except that there were three lots—some well watered, others watered only when it was necessary to keep them from dying, and some others grown outside at a temperature considerably lower than that in the greenhouse. The following table gives the results:

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TABLE 5. SHOWING EFFECT OF CONDITION OF GROWTH ON SAP DENSITY AND HARDINESS OF TOMATOES.

Treatment	Date	Temperature	Number of Plants	Result of Freezing	Depression
Out of doors....	Apr. 29,'11	−2	4	All dead.....	0.73
Greenhouse, wet	Apr. 29,'11	−2	4	Leaves all dead; stems slightly injured.....	0.84
Greenhouse, dry	Apr. 29,'11	−2	4	Uninjured except very young leaves.....	1.16
Out of doors....	May 2,'11	−2	4	All dead (larger and stockier).....	.73
Greenhouse, wet	May 2,'11	−2	4	Leaves dead; lower stems alive.....	0.84
Greenhouse, dry	May 2,'11	−2	4	Only few leaves killed	1.16
Out of doors....	May 4,'11	−2.5	4	Foliage and upper one-third stems killed....	0.73
Greenhouse, wet	May 4,'11	−2.5	4	All killed.....	0.84
Greenhouse, dry	May 4,'11	−2.5	4	Leaves killed; stems uninjured.....	1.16
Out of doors....	May 6,'11	−2.5	4	Leaves killed; stems uninjured.....	.73
Greenhouse, wet	May 6,'11	−2.5	4	Leaves dead; upper one-third stems dead.....	0.84
Greenhouse, dry	May 6,'11	−2.5	4	Foliage and growing tips of three plants dead; one plant uninjured.....	1.16

Contrary to what might be expected, those tomato plants grown in the greenhouse but watered sparingly were more hardy than those grown outside; also the depression was greater. The results in this table again indicate that as the depression is lowered, plants are made more hardy.

Cabbage, kale and turnips were each grown in the greenhouse some watered well and others with water withheld except when it was necessary to keep the plants alive, while others were grown out of doors. The following table give results, and depressions for these plants.

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TABLE 6. SHOWING INFLUENCE OF CONDITION OF GROWTH ON SAP DENSITY AND HARDINESS.

Treatment	Date	Temperature	Number of Leaves	Percentage Killed	Percentage Killed and Partly Killed	Depression
CABBAGE						
Out of doors	Nov. 2,'11	−5.5	3	0	0	....
Greenhouse, dry	Nov. 2,'11	−5.5	3	0	0	....
Greenhouse, wet	Nov. 2,'11	−5.5	3	33.4	33.4	....
Out of doors	Nov. 4,'11	−6	3	0	0	....
Greenhouse, dry	Nov. 4,'11	−6	3	0	0	....
Greenhouse, wet	Nov. 4,'11	−6	3	66.7	66.7	....
Out of doors	Nov. 17,'11	−6.5	4	0	0	....
Greenhouse, dry	Nov. 17,'11	−6.5	5	100	100	....
Greenhouse, wet	Nov. 17,'11	−6.5	5	100	100	....
Out of doors	Dec. 9,'11	−5	4	0	0	....
Greenhouse, dry	Dec. 9,'11	−5	4	100	100	....
Greenhouse, wet	Dec. 9,'11	−5	4	100	100	....
Out of doors	Dec. 9,'11	−5	4	0	0	....
Greenhouse, dry	Dec. 9,'11	−5	4	100	100	....
Greenhouse, wet	Dec. 9,'11	−5	4	100	100	....
Out of doors	Dec. 13,'11	−4	3	0	0	1.18
Greenhouse, dry	Dec. 13,'11	−4	3	0	0	.90
Greenhouse, wet	Dec. 13,'11	−4	2	100	100	.99
Out of doors, average				0	0	....
Greenhouse, dry; average				50	50	....
Greenhouse, wet; average				83.3	83.3	....
TURNIPS						
Out of doors	Nov. 2,'11	−5.5	3	0	100	....
Greenhouse, dry	Nov. 2,'11	−5.5	3	100	100	....
Greenhouse, wet	Nov. 2,'11	−5.5	3	100	100	....
Out of doors	Nov. 4,'11	−6	3	0	0	....
Greenhouse, dry	Nov. 4,'11	−6	3	100	100	....
Greenhouse, wet	Nov. 4,'11	−6	3	100	100	....
KALE						
Out of doors, coldframe	Dec. 8,'11	−6.5	2	50	50	....
Out of doors, bed	Dec. 8,'11	−6.5	3	0	0	....
Greenhouse, dry	Dec. 8,'11	−6.5	3	100	100	....
Greenhouse, wet	Dec. 8,'11	−6.5	3	100	100	....
LETTUCE						
Out of doors	Mar. 9,'13	−3.5	8	0	25	.900
Greenhouse	Mar. 9,'13	−3.5	9	83.3	100.0	.867
Out of doors	Mar. 29,'13	−5	18	0	27.7	.900
Greenhouse	Mar. 29,'13	−5	16	68.7	93.7	.867
Out of doors	Apr. 30,'13	−3.5	24	0	33.3	.920
Greenhouse	Apr. 30,'13	−3.5	32	18	48	.740
Average, lettuce out of doors				0.0	28.7	.907
Average, lettuce greenhouse				56.6	80.1	.825

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Depressions were not determined for each day's f account of the limited number of plants, but depression other lots grown under the same conditions show simil Thus a set taken January 6, 1912, showed the following d Plants grown out of doors, depression, 1.470; plants gr greenhouse with limited water supply, depression, 1.0 grown in the greenhouse with abundant water supply, .990. Here again increased sap density is accompanied hardiness, and in this case the plants with the greatest d also the ones which grew most rapidly.

In all of these cases any treatments that increased t of the sap lowered the killing temperature. It should be ever, that in most cases where the density of the sap ha creased, the growth of the plants has been checked so we c positively that a treatment has increased the hardiness, density of the sap, since it could probably be due to the sm or some other differences in the conditions of the protoplas ever, cabbage and kale were exceptions to this and actu more rapidly out of doors and yet had more dense sap and hardy. In order to test the effect of increased sap density ness under conditions where this effect on growth would nated, plants of tomato, cabbage, lettuce, kale and cow grown under like conditions. Then the plants were pulled washed clean and placed in sugar solutions and in potas ride and other solutions of varying strengths as shown in with the results to be seen in the following table.

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TABLE 7. SHOWING INFLUENCE OF ABSORBED SOLUTIONS ON HARDINESS.

Roots 24 hrs. in solutions of strength measured by freezing points given below	Date	Temperature	Number of Leaves	Percentage Killed	Percentage Killed and Partly Killed	Depression
TOMATOES						
Glucose (.460)	July 27,'11	-3.0	84	63.0	67.6	0.785
Cane Sugar (.435)	July 27,'11	-3.0	60	83.3	88.3	0.925
Glycerine (.430)	July 27,'11	-3.0	70	72.8	88.5	1.070
Potassium Nitrate (.463)	July 27,'11	-3.0	57	100.0	100.0	0.880
Water	July 27,'11	-3.0	65	100.0	100.0	0.700
Glucose (.460)	July 27,'11	-2.0	60	0.0	0.0	0.785
Cane Sugar (.435)	July 27,'11	-2.0	57	24.5	56.1	0.925
Glycerine (.430)	July 27,'11	-2.0	44	50.0	53.6	1.070
Potassium Nitrate (.463)	July 27,'11	-2.0	46	60.8	73.9	0.880
Water	July 27,'11	-2.0	48	62.5	72.9	0.700
Glucose (.460)	July 28,'11	-3.5	48	91.6	95.8	0.785
Cane Sugar (.435)	July 28,'11	-3.5	47	89.3	95.7	0.925
Glycerine (.430)	July 28,'11	-3.5	55	94.5	100.0	1.070
Potassium Nitrate (.463)	July 28,'11	-3.5	53	98.1	100.0	0.880
Water	July 28,'11	-3.5	43	76.7	100.0	0.70
Glucose (.460)	July 28,'11	-2.5	47	59.5	70.0	0.785
Cane Sugar (.435)	July 28,'11	-2.5	58	13.8	17.2	0.925
Glycerine (.430)	July 28,'11	-2.5	52	9.6	44.2	1.070
Potassium Nitrate (.463)	July 28,'11	-2.5	43	83.7	100.0	0.880
Water	July 28,'11	-2.5	42	11.9	16.6	0.700
Glucose (.460)	July 28,'11	-3	33	69.7	69.7	0.785
Cane Sugar (.435)	July 28,'11	-3	37	18.9	35.1	0.925
Glycerine (.430)	July 28,'11	-3	44	61.3	61.3	1.070
Potassium Nitrate (.463)	July 28,'11	-3	46	100.0	100.0	0.880
Water	July 28,'11	-3	42	66.6	66.6	0.700
Glucose (.460), average				56.8	60.6	0.785
Cane Sugar (.435), average				45.9	58.5	0.925
Glycerine (.430), average				57.6	69.5	1.070
Potassium Nitrate (.463), average				88.5	94.6	0.880
Water, average				63.5	71.2	0.700

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Roots 24 hrs. in solutions of strength measured by freezing points given below	Date	Temperature	Number of Leaves	Percentage Killed	Percentage Killed and Partly Killed	Depression
TOMATOES Treated 18 hrs.						
Water	June 28,'13	−2.5	20	50.0	65.0	.698
Potassium Chloride (.775)	June 28,'13	−2.5	19	0.0	0.0	1.103
Ammonium Chloride (.360)	June 28,'13	−2.5	15	66.7	93.3	.863
Glycerine (2.820)	June 28,'13	−2.5	27	0.0	0.0	2.083
Water	June 28,'13	−3.0	16	100.0	100.0	.698
Potassium Chloride (.775)	June 28,'13	−3.0	17	0.0	35.3	1.103
Ammonium Chloride (.360)	June 28,'13	−3.0	16	68.7	77.4	.863
Cane Sugar (.677)	June 28,'13	−3.0	30	53.3	76.6	1.053
CABBAGE						
Cane Sugar (0.77)	Aug. 24,'11	−4.0	3	100.0	100.0	1.230
Glucose (0.44)	Aug. 24,'11	−4.0	3	100.0	100.0	1.190
Glycerine (0.66)	Aug. 24,'11	−4.0	3	66.6	66.6	1.270
Potassium Chloride (0.73)	Aug. 24,'11	−4.0	3	0.0	0.0	1.525
Ammonium Chloride (0.51)	Aug. 24,'11	−4.0	3	100.0	100.0	1.195
Water	Aug. 24,'11	−4.0	3	66.6	66.6	1.080
Cane Sugar (0.77)	Aug. 26,'11	−3.5	3	66.6	66.6	1.230
Glucose (0.44)	Aug. 26,'11	−3.5	3	66.6	66.6	1.190
Glycerine (0.66)	Aug. 26,'11	−3.5	3	66.6	66.6	1.270
Potassium Chloride (0.73)	Aug. 26,'11	−3.5	3	0.0	0.0	1.525
Ammonium Chloride (0.51)	Aug. 26,'11	−3.5	3	100.0	100.0	1.195
Water	Aug. 26,'11	−3.5	3	100.0	100.0	1.080
Cane Sugar (0.77)	Aug. 31,'11	−5.5	3	66.6	66.6	1.230
Glucose (0.44)	Aug. 31,'11	−5.5	3	33.3	33.3	1.190
Glycerine (0.66)	Aug. 31,'11	−5.5	3	0.0	0.0	1.270
Potassium Chloride (0.73)	Aug. 31,'11	−5.5	3	0.0	0.0	1.525
Ammonium Chloride (0.51)	Aug. 31,'11	−5.5	3	66.6	66.6	1.195
Water	Aug. 31,'11	−5.5	3	0.0	0.0	1.080
Cane Sugar (0.77), average				77.7	77.7	1.230
Glucose (0.44), average				66.6	66.6	1.190
Glycerine (0.66), average				44.4	44.4	1.270
Potassium Chloride (0.73), average				0.0	0.0	1.525
Ammonium Chloride (0.51), average				88.8	88.8	1.195
Water, average				55.5	55.5	1.080

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Roots 24 hrs. in solutions of strength measured by freezing points given below	Date	Temperature	Number of Leaves	Percent-Age Killed	Percentage Killed and Partly Killed	Depression
CABBAGE (later freezing)						
No treatment	July 1,'13	−4.0	5	80.0	100.0	.780
Potassium Chloride (.775)	July 1,'13	−4.0	5	40.0	100.0	1.145
Glycerine (2.82)	July 1,'13	−4.0	5	20.0	80.0	1.780
Ammonium Chloride (.360)	July 1,'13	−4.0	5	100.0	100.0	.950
COWPEAS						
Cane Sugar (1.570)	Sept. 1,'11	−3.0	3	0.0	0.0	1.230
Glucose (1.740)	Sept. 1,'11	−3.0	3	100.0	100.0	1.250
Glycerine (1.575)	Sept. 1,'11	−3.0	3	0.0	0.0	1.160
Potassium Chloride (0.730)	Sept. 1,'11	−3.0	3	100.0	100.0	1.130
Ammonium Chloride (0.725)	Sept. 1,'11	−3.0	3	66.6	66.6	1.140
Water	Sept. 1,'11	−3.0	3	33.3	33.3	.870
Cane Sugar (1.570)	Sept. 9,'11	−3.0	3	0.0	0.0	1.230
Glucose (1.740)	Sept. 9,'11	−3.0	3	33.3	66.6	1.250
Glycerine (1.575)	Sept. 9,'11	−3.0	3	0.0	0.0	1.160
Potassium Chloride (0.730)	Sept. 9,'11	−3.0	3	0.0	0.0	1.130
Ammonium Chloride (0.725)	Sept. 9,'11	−3.0	3	33.3	100.0	1.140
Water	Sept. 9,'11	−3.0	3	100.0	100.0	.870
Cane Sugar (1.570)	Sept. 9,'11	−3.5	3	0.0	0.0	1.230
Glucose (1.740)	Sept. 9,'11	−3.5	3	66.6	66.6	1.250
Glycerine (1.575)	Sept. 9,'11	−3.5	3	0.0	0.0	1.160
Potassium Chloride (0.730)	Sept. 9,'11	−3.5	3	66.6	66.6	1.130
Ammonium Chloride (0.725)	Sept. 9,'11	−3.5	3	66.6	66.6	1.140
Water	Sept. 9,'11	−3.5	3	33.3	33.3	.870
Cane Sugar (1.570)	Aug. 29,'11	−3	3	0.0	0.0	1.230
Glucose (1.740)	Aug. 29,'11	−3	3	0.0	0.0	1.250
Glycerine (1.575)	Aug. 29,'11	−3	3	0.0	0.0	1.160
Potassium Chloride (0.730)	Aug. 29,'11	−3.0	3	100.0	100.0	1.130
Ammonium Chloride (0.725)	Aug. 29,'11	−3.0	3	0.0	0.0	1.140
Water	Aug. 29,'11	−3.0	3	100.0	100.0	.870
Cane Sugar (1.570), average				0.0	0.0	1.230
Glucose (1.740), average				49.9	58.3	1.250
Glycerine (1.575), average				0.0	0.0	1.160
Potassium Chloride (0.730), average				66.6	66.6	1.130
Ammonium Chloride (0.725), average				41.6	58.3	1.140
Water, average				66.6	66.6	.870

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Roots 24 hrs. in solutions of strength measured by freezing points given below	Date	Temperature	Number of Leaves	Percentage Killed
KALE				
Cane Sugar (.677)	June 25,'13	-3.0	5	0.0
Glycerine (2.820)	June 25,'13	-3.0	5	20.0
Potassium Chloride (.775)	June 25,'13	-3.0	5	60.0
Ammonium Chloride (.360)	June 25,'13	-3.0	5	80.0
Water	June 25,'13	-3.0	5	80.0
Cane Sugar (.677)	July 9,'13	-1.5	5	40.0
Glycerine (2.820)	July 9,'13	-1.5	5	0.0
Potassium Chloride (.775)	July 9,'13	-1.5	5	0.0
Ammonium Chloride (.360)	July 9,'13	-1.5	5	60.0
Water	July 9,'13	-1.5	5	60.0
LETTUCE				
Cane Sugar (.677)	July 2,'13	-2.5	5	0.0
Glycerine (2.820)	July 2,'13	-2.5	5	0.0
Potassium Chloride (.775)	July 2,'13	-2.5	5	20.0
Ammonium Chloride (.360)	July 2,'13	-2.5	5	40.0
Water	July 2,'13	-2.5	5	40.0
Cane Sugar (.677)	July 2,'13	-3.5	5	20.0
Glycerine (2.820)	July 2,'13	-3.5	5	0.0
Potassium Chloride (.775)	July 2,'13	-3.5	5	40.0
Ammonium Chloride (.360)	July 2,'13	-3.5	5	60.0
Water	July 2,'13	-3.5	5	60.0
Cane Sugar (.677)	July 8,'13	-3.0	5	0.0
Glycerine (2.820)	July 8,'13	-3.0	5	0.0
Potassium Chloride (.775)	July 8,'13	-3.0	5	0.0
Ammonium Chloride (.360)	July 8,'13	-3.0	5	60.0
Water	July 8,'13	-3.0	5	20.0

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Here again the hardiness has been increased by increasing the ensity of the sap. Of course it should be admitted that even here here is a possibility that some actual change in the protoplasm has aken place by treating it with these solutions. Glycerine has been nost effective in increasing the sap density of the tissue and in increasing the resistance to cold. It is interesting to observe that in he case of cabbage, the sap density and the hardiness were more reatly increased with salts like potassium chloride than with cane ugar, while in the case of tomatoes, sugar was taken up in larger uantities and caused a greater increase in hardiness.

According to the theory of Gorke, if killing is due to the salting out of proteids, we should expect the taking up of sugar to increase he hardiness but should not expect that result to follow the taking p of salts. The salts that most readily precipitate certain proteids re ammonium sulphate and zinc sulphate. When roots of tomato lants were kept for twenty-four hours in solutions of a molecular oncentration as great as those used in the table above, the hardiess of the leaves was not reduced, and zinc sulphate seemed to increase the hardiness. These results are not in accord with Gorke's heory. Potassium nitrate does not increase the hardiness as other ubstances do. Thus tomato plants with their roots kept in potasium nitrate solution of about the same molecular concentration as he solutions used above, seemed to be killed more easily than when he roots were kept in pure water, and corn plants so treated cerainly were killed more easily. This lack of protective action is robably due to the high eutectic point of the potassium nitrate ince it would precipitate out before the killing temperature of the issue is reached.

Apple and peach blossoms were cut from the twigs in such a ay that a considerable area of cortex and sap wood adhered to the tem, and these were inserted in solutions of varying strengths of ugar and glycerine and later frozen. The following table gives the esults:

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TABLE 8. SHOWING INFLUENCE OF ABSORBED SOLUTIONS ON HARDINESS OF YOUNG FRUITS.

Kind of Fruit	Treatment	Date	Temperature	Number of Fruits	Percentage Killed
Rice's Seedling peach blossoms	Fresh	Apr. 15,'11	-3	43	70.0
Rice's Seedling peach blossoms	Cane sugar (2.250)	Apr. 15,'11	-3	34	3.0
Rice's Seedling peach blossoms	Glycerine (2.560)	Apr. 15,'11	-3	45	22.0
Rice's Seedling peach blossoms	Water	Apr. 15,'11	-3	50	100.0
Rice's Seedling peach blossoms	Cane sugar (2.250)	Apr. 20,'11	-3	13	61.0
Rice's Seedling peach blossoms	Glycerine (2.560)	Apr. 20,'11	-3	22	36.0
Rice's Seedling peach blossoms	Water	Apr. 20,'11	-3	15	66.0
Rice's Seedling peach blossoms, petals just fallen	Cane sugar (2.250)	Apr. 20,'11	-3	24	81.0
Rice's Seedling peach blossoms, petals just fallen	Glycerine (2.560)	Apr. 20,'11	-3	21	81.0
Rice's Seedling peach blossoms, petals just fallen	Water	Apr. 20,'11	-3	13	58.0
Peaches in husk	Fresh	May 4,'11	-3	..	12.2
Peaches in husk	Cane sugar (2.250)	May 4,'11	-3	..	8.7
Peaches in husk	Glycerine (2.560)	May 4,'11	-3	..	6.0
Peaches in husk	Water	May 4,'11	-3	..	70.0
Peaches 2-5 in. in dia.	Fresh	May 11,'11	-3.9	21	24.0
Peaches 2-5 in. in dia.	Cane sugar (2.250)	May 11,'11	-3.9	20	35.0
Peaches 2-5 in. in dia.	Glycerine (2.560)	May 11,'11	-3.9	25	4.0
Peaches 2-5 in. in dia.	Water	May 11,'11	-3.9	18	38.0
Apple buds showing pink	Canesugar (2.250)	Apr. 24,'11	-3	31	61.0
Apple buds showing pink	Glycerine (2.560)	Apr. 24,'11	-3	30	13.0
Apple buds showing pink	Water	Apr. 24,'11	-3	25	96.0
Apple buds nearly open	Fresh	Apr. 24,'11	-3	30	100.0
Apple buds nearly open	Cane sugar (2.250)	Apr. 24,'11	-3	40	60.0
Apple buds nearly open	Glycerine (2.560)	Apr. 24,'11	-3	32	0.0
Apple buds nearly open	Water	Apr. 24,'11	-3	45	100.0
Apple buds partly open	Cane sugar (2.250)	Apr. 26,'11	-3	34	3.0
Apple buds partly open	Glycerine (2.560)	Apr. 26,'11	-3	34	61.0

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Kind of Fruit	Treatment	Date	Temperature	Number of Fruits	Percentage Killed
Apple buds partly open	Water.....	Apr. 26,'11	-3	23	100.0
Apple blossoms open...	Fresh.....	Apr. 26,'11	-3	27	4.0
Apple blossoms open...	Cane sugar (2.250)....	Apr. 26,'11	-3	50	0.0
Apple blossoms open...	Glycerine (2.560)....	Apr. 26,'11	-3	50	14.0
Apple blossoms open...	Water.....	Apr. 26,'11	-3	37	92.0
Apple blossoms, petals just fallen.....	Fresh.....	May 4,'11	-3	28	36.0
Apple blossoms, petals just fallen.....	Cane sugar (2.250)....	May 4,'11	-3	50	4.0
Apple blossoms, petals just fallen.....	Glycerine (2.560)....	May 4,'11	-3	44	4.0
Apple blossoms, petals just fallen.....	Water.....	May 4,'11	-3	43	72.0
Apples just after petals fall.....	Fresh.....	May 11,'11	-3.9	58	35.0
Apples just after petals fall.....	Cane sugar (2.250)....	May 11,'11	-3.9	83	9.6
Apples just after petals fall.	Glycerine (2.560)....	May 11,'11	-3.9	76	3.0
Apples just after petals fall.....	Water.....	May 11,'11	-3.9	66	79.0
Apples 1-3 in. in dia....	Fresh.....	May 11,'11	-3.9	17	41.0
Apples 1-3 in. in dia....	Cane sugar (2.250)....	May 11,'11	-3.9	27	20.0
Apples 1-3 in. in dia....	Glycerine (2.560)....	May 11,'11	-3.9	31	6.4
Apples 1-3 in. in dia....	Water.....	May 11,'11	-3.9	28	100.0
Wild Goose plums.....	Cane sugar (2.250)....	May 11,'11	-3.9	25	4.0
Wild Goose plums.....	Glycerine (2.560)....	May 11,'11	-3.9	25	0.0
Wild Goose plums.....	Water.....	May 11,'11	-3.9	21	16.0
Average, Cane sugar (2.50).....					26.9
Average, Glycerine (2.560).....					19.2
Average, Water.....					75.9

It will be seen again that absorbing the solutions increased the resistance to cold. Depressions were not taken because it would require too many fruits treated in this way. However, it is safe to assume that the sap density was increased as it certainly was with the fruits used for the next table.

During the spring of 1913, twigs containing peaches, apples and cherries were placed with the ends in glycerine solution, sugar

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solution and in pure water. These fruits absorbed the solutions readily as the depression data following the table will show. The following table gives the result of freezing these fruits:

TABLE 9. SHOWING EFFECT OF ABSORBED SOLUTIONS ON HARDINESS OF BLOSSOMS AND YOUNG FRUITS ON TWIGS.

Kind of Fruit	Treatment	Date	Temperature	Number of Fruits	Percentage Killed
Twigs containing very young Rareripe peaches	Water 23 hrs.	Apr. 28,'13	-5	56	55.3
Twigs containing very young Rareripe peaches	20% Cane sugar 23 hrs.	Apr. 28,'13	-5	77	29.7
Twigs containing very young Rareripe peaches	10% Glycerine 23 hrs.	Apr. 28,'13	-5	51	11.8
Twigs containing very young Rareripe peaches	Fresh from tree.	Apr. 28,'13	-5	69	49.3
Twigs containing young Hiley peaches	10% Glycerine 20 hrs.	May 1,'13	-4	65	1.5
Twigs containing young Hiley peaches	Fresh from tree.	May 1,'13	-4	76	1.3
Twigs containing Lewis peaches	Water 21 hrs.	May 17,'13	-4	22	45.5
Twigs containing Lewis peaches	10% Glycerine 21 hrs.	May 17,'13	-4	35	11.4
Twigs containing Lewis peaches	Wilted 6 hrs.	May 17,'13	-4	22	45.5
Twigs containing Lewis peaches	Fresh from tree.	May 17,'13	-4	23	43.5
Twigs containing Bernard peaches	10% Glycerine 22 hrs.	May 21,'13	-4.2	51	13.7
Twigs containing Bernard peaches	Wilted 5 hrs.	May 21,'13	-4.2	53	69.8
Twigs containing Bernard peaches	Fresh from tree.	May 21,'13	-4.2	34	61.8
Twigs containing open Rome Beauty apple blossoms	20% Cane sugar solution 20 hrs.	May 1,'13	-4	96	15.6
Twigs containing open Rome Beauty apple blossoms	10% Glycerine 20 hrs.	May 1,'13	-4	108	13.8
Twigs containing open Rome Beauty apple blossoms	Fresh from tree	May 1,'13	-4	83	40.0

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Kind of Fruit	Treatment	Date	Temperature	Number of Fruits	Percentage Killed
Twigs containing Jonathan apples.....	Water 21 hrs.....	May 17,'13	−4	27	70.4
Twigs containing Jonathan apples.....	10% Glycerine 21 hrs.....	May 17,'13	−4	32	43.8
Twigs containing Jonathan apples.....	Wilted 6 hrs.....	May 17,'13	−4	37	86.5
Twigs containing Jonathan apples.....	Fresh from tree.....	May 17,'13	−4	19	68.4
Twigs containing Dyehouse cherries ..	Water 21 hrs.....	May 17,'13	−4	42	30.9
Twigs containing Dyehouse cherries.....	10% Glycerine 21 hrs.....	May 17,'13	−4	43	13.9
Twigs containing Dyehouse cherries....	Wilted 6 hrs.....	May 17,'13	−4	36	48.8
Twigs containing Dyehouse cherries....	Fresh from tree.....	May 17,'13	−4	15	66.6
Twigs containing Dyehouse cherries.....	10% Glycerine 22 hrs.....	May 21,'13	−4.2	39	5.1
Twigs containing Dyehouse cherries....	Wilted 5 hrs.....	May 21,'13	−4.2	56	21.4
Twigs containing Dyehouse cherries....	Fresh from trees.....	May 21,'13	−4.2	38	39.5
Average percentage killed, fresh.....					48.8
Average percentage killed, glycerine.....					14.4

Since it requires a large number of blooms or young fruits to furnish sap for a freezing point determination, only one such determination was made. This resulted as follows, using fruits treated just as they were for freezing:

	Depression degrees
Cherries fresh from tree.....	0.905
Cherries from twigs with ends in glycerine sixteen hours.....	1.180
Cherries wilted five hours.....	1.075
Peaches fresh from tree.....	.965
Peaches from twigs with ends in glycerine sixteen hours.....	1.230
Peaches wilted five hours.....	1.085
Apples from twigs with ends in glycerine thirty hours.....	1.408
Apples from twigs with ends in water thirty hours.....	1.335
Apples from twigs with ends in cane sugar thirty hours.....	1.530
Apples from twigs with ends in glycerine forty-eight hours.....	1.417
Apples from twigs with ends in water forty-eight hours.....	1.030
Apples from twigs with ends in cane sugar forty-eight hours.....	1.160

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It seems then practically certain that one is justified in assuming that the fruits in the above freezing table had their osmotic strength increased as much by taking up the glycerine and sugar solutions as this depression data indicates. The fruits that had absorbed the glycerine were apparently fully turgid while the wilted fruits were very flaccid. It is certain that wilting could have had little part in increasing the sap density of the fruits absorbing the glycerine. It may be suggested by some that possibly the tissue could not absorb these rather strong solutions as rapidly as it could absorb water, so there might be some wilting to cause the greater hardiness attributed to the increased sap density. Observations were made on this point in all cases and rarely was there any signs of wilting in the frozen tissues referred to. In most cases, plants which were wilted until they were very limp before treating as described above were still less hardy than those absorbing the solutions. It is thus certain that the greater hardiness of the tissue absorbing the various solutions was not due to wilting. The effect of wilting on tissue is discussed later in this paper.

Reducing Sap Density by Shading. The sap density of leaves is known to increase from morning to afternoon. Leaves shaded usually have a lower sap density than those in the light. It was thought that this would be another good means of testing the effect of sap density on hardiness. The following plants (or twigs containing leaves) were shaded for twenty-four hours and taken for freezing in early afternoon along with plants under similar conditions, except that they were in full sunlight. The results are shown in Table 10:

TABLE 10. SHOWING EFFECT OF SHADING ON SAP DENSITY AND RESISTANCE TO FREEZING.

Material	Treatment	Date	Temperature	Number of Plants	Percentage All Killed	Percentage Total Leaf Area Killed	Depression
Cowpeas.....	Shaded....	June 14,'13	−4	3	0.0	90.0	.852
Cowpeas.....	Not shaded	June 14,'13	−4	2	100.0	100.0	.980
Cowpeas.....	Shaded....	June 14,'13	−3	3	0.0	90.0	.852
Cowpeas.....	Not shaded	June 14,'13	−3	3	0.0	22.0	.980
Cowpeas.....	Shaded....	June 17,'13	−3	3	66.6	66.6	.855
Cowpeas.....	Not shaded	June 17,'13	−3	4	25.0	81.2	.947

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Material	Treatment	Date	Temperature	Number of Plants	Percentage All Killed	Percentage Total Leaf Area Killed	Depression
White corn.....	Shaded....	June 13,'13	−4	15	....	99.0	.728
White corn....	Not shaded	June 13,'13	−4	12	....	99.0	.888
Corn.....	Shaded....	June 17,'13	−3	7	18.8	55.0	.835
Corn.....	Not shaded	June 17,'13	−3	7	0.0	25.0	1.035
Tomato leaflets.	Shaded....	June 19,'13	−2	34	38.2	65.3	.708
Tomato leaflets.	Not shaded	June 19,'13	−2	51	0.0	0.0	.848
Tomato leaflets.	Shaded....	June 19,'13	−3.5	35	100.0	100.0	.708
Tomato leaflets.	Not shaded	June 19,'13	−3.5	36	47.2	63.9	.848
Tomato leaves..	Shaded....	June 25,'13	−4	6	50.0	87.0	.595
Tomato leaves..	Not shaded	June 25,'13	−4	5	40.0	55.0	.752
Kale leaves.....	Shaded....	June 25,'13	−3.5	5	100.0	100.0	.660
Kale leaves.....	Not shaded	June 25,'13	−3.5	5	40.0	70.0	.790
Lettuce leaves...	Shaded....	June 25,'13	−3.5	6	100.0	100.0	.490
Lettuce leaves..	Not shaded	June 25,'13	−3.5	6	100.0	100.0	.590
Lettuce leaflets.	Shaded....	June 21,'13	−2.8	13	23.0	50.0	.570
Lettuce leaflets.	Not shaded	June 21,'13	−2.8	17	0.0	1.4	.740
Lettuce leaflets.	Shaded....	June 21,'13	−4	18	100.0	100.0	.570
Lettuce leaflets.	Not shaded	June 21,'13	−4	17	41.1	66.1	.740
Red Rock Cabbage leaflets	Shaded....	June 20,'13	−3.5	14	0.0	19.6	.860
Red Rock Cabbage leaflets..	Not shaded	June 20,'13	−3.5	14	7.1	7.1	.955
Red Rock Cabbage leaflets..	Shaded....	June 20,'13	−4.5	15	53.3	83.3	.860
Red Rock Cabbage leaflets..	Not shaded	June 20,'13	−4.5	12	50.0	77.0	.955
Cabbage leaflets	Shaded....	June 25,'13	−3.5	36	52.8	77.7	.680
Cabbage leaflets	Not shaded	June 25,'13	−3.5	38	36.8	63.1	.875
Early Harvest apple twigs and leaves....	Shaded....	June 28,'13	−4	39	0.0	35.9	1.975
Early Harvest apple twigs and leaves....	Not shaded	June 28,'13	−4	42	0.0	6.8	2.438
Early Harvest apple twigs and leaves....	Shaded....	June 28,'13	−5	47	34.0	70.0	1.975
Early Harvest apple twigs and leaves...	Not shaded	June 28,'13	−5	50	0.0	48.5	2.438
Early Harvest apple twigs and leaves....	Shaded....	July 1,'13	−4	34	0.0	19.8	1.930
Early Harvest apple twigs and leaves....	Not shaded	July 1,'13	−4	40	0.0	11.2	2.252
Early Harvest apple twigs and leaves....	Shaded....	July 1,'13	−5.5	38	0.0	40.1	1.930

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Material	Treatment	Date	Temperature	Number of Plants	Percentage All Killed	Percentage Total Leaf Area Killed	Depression
Early Harvest apple twigs and leaves....	Not shaded	July 1,'13	-5.5	46	0.0	16.3	2.252
Pear twigs and leaves.....	Shaded....	June 28,'13	-4	25	24.0	74.0	
Pear twigs and leaves.....	Not shaded	June 28,'13	-4	28	0.0	36.6	
Pear twigs and leaves.....	Shaded....	June 28,'13	-5	25	80.0	95.0	
Pear twigs and leaves.....	Not shaded	June 28,'13	-5	26	15.4	63.4	
Average, Shaded, excluding pears.....					33.5	71.01	.985
Average, Not shaded, excluding pears.....					30.9	48.61	1.173

In practically all cases the cortex, cambium and sap wood of the twigs were injured rather severely, and in all cases the injury was worse with the shaded twigs.

In the case of the pear twigs and leaves sufficient sap for depression determination could not be secured.

Material	Treatment	Date	Temperature	Number of Plants	Percentage All Killed	Percentage Total Leaf Area Killed	Depression
Labrusca grape leaves.....	Shaded 38 hours .	July 8,'13	-3	18	27.2	54.2	.695
Labrusca grape leaves.....	Not shaded	July 8,'13	-3	17	35.3	58.8	.733
Labrusca grape leaves.....	Shaded 38 hours..	July 8,'13	-4.5	22	100.	100.	.695
Labrusca grape leaves.....	Not shaded	July 8,'13	-4.5	17	76.4	94.1	.733
Labrusca grape leaves.....	Shaded 22 hours..	July 10,'13	-3.5	10	0.0	2.5	.755
Labrusca grape leaves.....	Not shaded	July 10,'13	-3.5	10	0.0	0.0	.920
Labrusca grape leaves.....	Shaded 22 hours..	July 10,'13	-4.5	10	30.0	30.0	.755
Labrusca grape leaves.....	Not shaded	July 10,'13	-4.5	10	0.0	15.0	.920
Labrusca grape leaves.....	Shaded 22 hours..	July 11,'13	-4	10	10.0	25.0	.835
Labrusca grape leaves.....	Not shaded	July 11,'13	-4	10	0.0	17.5	1.085
Labrusca grape leaves.....	Shaded 22 hours..	July 11,'13	-5.5	10	60.0	80.0	.835
Labrusca grape leaves.....	Not shaded	July 11,'13	-5.5	10	40.0	52.5	1.085
Average, shaded.....					34.53	48.61	.761
Average, not shaded.....					25.29	39.65	.912

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While the differences are not large, it will be seen that the sap density of the shaded plants is uniformly lower and the killing greater.

Ohlweiler[1] at the Missouri Botanical Garden seemed to find some relation between the density of the sap of different plant species and their resistance to cold. This is true especially in the case of the different species of magnolia, where the leaf structure of species with dense sap and of those with dilute sap is similar, so there would not be this influence involved to modify the results.

From the beginning of these experiments, observations have been made in autumn as to plants killed by the various early frosts and freezing point determinations were made from leaves of these to see if there is to be found any relation between hardiness and sap density. In the following table the plants are listed as nearly as could be determined according to hardiness, the most tender first, though it is certain that almost any of the plants could be changed two or three places in the succession and be as accurately placed in order of hardiness. The depressions are also given. The leaves for the depressions were all taken in the morning as soon as the dew was off so they would be equally turgid.

TABLE 11. SHOWING THE RELATIVE HARDINESS OF GROWING PLANTS COMPARED WITH THEIR RELATIVE SAP DENSITY.

Plant	Depression
Morning-Glory (Ipomoea purpurea)	.920
Coleus (C. Blumei)	.428
Sweet Potato (Ipomoea Batatas)	.96
Moon Vine (Ipomoea Bona-Nox)	.863
Watermelon (Citrullus vulgaris)	.882
Cantaloupe (Cucumis Melo)	.588
Cucumber (Cucumis sativus)	.585
Caladium (Colocasia antiquorum)	.745
Pumpkin (Cucurbita Pepo)	.785
Tomato (Lycopersicum esculentum)	.832
Lantana (L. Camara)	.962
Dahlia (Dahlia variabilis)	.711
Blue Salvia (Salvia patens)	1.025
Red Salvia (Salvia splendens)	.765
Rose Geranium (Pelargonium graveolens)	1.835
Geranium (Pelargonium Hortorum)	1.075
Eggplant (Solanum Melongena)	.805
Alternanthera (Telanthera versicolor)	1.058
Periwinkle (Vinca major)	1.20
Ageratum (A. conyzoides)	1.055
Chard (Beta vulgaris var. Cycla)	.805
Celery (Apium graveolens)	1.442

[1]23d. Anl. Rpt. Mo. Bot. Gard. 1912, pp. 101-31. (Bibl. 87).

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Plant	Depressi
Gaillardia (G. pulchella)	.803
Chrysanthemum (C. Sinense)	1.955
Sedum spectabile	.575
Dandelion (Taraxacum officinale)	.975
Dock (Rumex crispus)	.997
Verbena (V. hybrida)	1.093
Hollyhock (Althaea rosea)	1.130
Horse-radish (Cochlearia Armoracia)	1.125
California Poppy (Eschscholzia Californica)	1.198
Clover (Trifolium pratense)	1.290
Violet (Viola odorata)	1.225
Cabbage (Brassica oleracea)	1.115
Plantain (Plantago major)	1.380
Strawberry (Fragaria Chiloensis)	1.865

All these depressions were determined as late in autumn a was possible to secure healthy tissue.

While it is evident that other factors than sap density influe the hardiness, yet it seems true that more of the very tender pla are found among those with slight depressions, and a majority the most hardy among those with greater density. It is poss that if a larger list of plants were obtained, this might not be tru

All of these tests have been made with succulent plants t kill at a temperature a few degrees below the freezing point. I true that some rather hardy plants like cabbage, kale, lettuce garden peas have been influenced in their killing temperature as gr ly as have plants like tomatoes and cowpeas that kill at a temp ture but slightly below the freezing point. However, in case of w ter wood, and buds that have developed great resistance to c by some sort of change, the problem would perhaps be differ This experiment started with the idea that it might be possibl increase the hardiness of buds of the peach in winter by increa the sap density through the use of fertilizers. Accordingly, p of peach trees at Dixon, Missouri, were fertilized with potass chloride at the rate of about 500 pounds to the acre, in the spr of 1907, 1908, and 1909. Plots receiving potassium chloride at rate of a little more than 500 pounds to the acre during the sea of 1906, 1907, 1908, and 1909, were located in the orchard of Ozark Orchard Company at Goodman, Missouri; and plots recei 480 pounds of potassium chloride to the acre, beginning March, 1 were located with the Ozark Fruit Farm Company, Brands Missouri. Potassium was used in these experiments because s experiments indicate that it is more readily taken into the cel become an osmotically active agent than most other mineral nu

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ents. In these orchards we have had opportunity to observe the results following cold periods that killed all the buds, cold periods that killed nearly all of them, and cold periods that left a fair crop, but in no case has there been any apparent effect on hardiness resulting from the use of potassium as a fertilizer. Twigs were secured from these plots in winter, spring, and summer and the density of the sap of the cortex determined by expressing the sap, and determining its freezing point, and no difference in the density could be detected between the plots fertilized with potassium and those receiving no fertilizer. These determinations could not be made with sap from buds because it could not be secured in sufficient quantities.

If it were possible to increase the sap density by accumulation in the cell of potassium or other materials applied to the soil, it could possibly affect the killing temperature of the bloom or young fruits or young growth in spring, even if it should have no effect on the killing temperature of the buds in their dormant condition. C. Dussere[1] observed apparently a slightly greater hardiness of young grape shoots on vines that had been fertilized with potassium than on vines that were not so fertilized. However, analyses failed to show the presence of more potassium in the wood of those vines receiving potassium fertilizer than in the wood of those that had not received fertilizer.

It may be said that on our peach plots we have had occasion to observe the effect of spring frosts on the bloom, as well as the effect of winter cold on the buds and have failed to observe any difference due to application of potassium to the soil.

Briefly summerizing all our results with the relation of sap density to hardiness, we are safe in concluding that, at least in case of plants not in a resting condition, a large amount of dissolved material either in the sap within the cell or in a solution surrounding the cell, will protect the cell from injury due to low temperature, to some extent at least. The protection where plants take the material into the cell seems to be much less than where the material in solution surrounds the cell, if the work of Maximow is confirmed.

The practical means of increasing hardiness seem to be very limited. Withholding water from plants in a plant bed will check the growth and thus increase the sap density and the hardiness. It was thought possible that with such plants as cabbage which take up potassium salts readily, watering in the plant bed with a solution of such salts would tend to increase their hardiness. However, cabbage and tobacco plants growing in a hotbed soil watered for eighteen

[1]Bul. Soc. Sci. Nat. 5, ser 48 (1912) No. 176, pp. 393. (Bibl. No. 31).

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days with a solution of potassium chloride ranging from 4.02 per cent normal at first to 16.08 per cent normal toward the last showed no increase in either sap density or hardiness over that of plants not so treated. It is only in sand cultures that we have been able to increase the sap density of plants by watering them with solutions.

If freezing to death results from too complete withdrawal of water from the protoplasm, then, even with winter buds and wood, if the sap density could be increased with some material that stays in solution at temperatures lower than that at which the tissue kills, it would hold more water in solution and thus lower the temperature at which a killing degree of desiccation is reached. Müller-Thurgau's measurements showed that a very large percentage of the water in plant tissue is in the form of ice. In case of winter resting tissue very little water can be left unfrozen at a killing temperature. If freezing to death of plant tissue results from desiccation, then very small quantities of water must be sufficient to protect the protoplasm in case of the more hardy plants. Some writers hold that since some plants continue to live after nearly all the water is frozen out, desiccation of the protoplasm can not be the explanation of death from low temperatures. Others seem to hold the opinion that the protective action of the sap solute ends at the freezing point of the sap. It should be remembered that so long as the temperature is above the eutectic point of any of the sap solute, there will be some water present in the liquid state. The number of degrees which the killing temperature is lowered by an increase in sap density should be greater with plants that kill at the lower temperatures. Thus in case of a solution containing a gram molecule of a non-electrolyte in a liter of water, the freezing point is -1.86° C. In case of a solution with one-half of a gram molecule to a liter of water, the freezing point would be -0.93° C. The following figures would evidently be approximately true:

Temperature	Fraction of water unfrozen, gram-molecule, in a liter of water	Fraction of water unfrozen, one-half a gram-molecule, in a liter of water
-0.93° C.	all	all
-1.86° C.	all	1/2
-3.72° C.	1/2	1/4
-7.44° C.	1/4	1/8
-14.88° C.	1/8	1/16
-29.76° C.	1/16	1/32

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This is, of course, assuming that the eutectic point of the solution is below these temperatures. It also assumes that the freezing point is lowered exactly in proportion to the molar concentration of the solution; that is, if there be four gram molecules in a liter of water, the freezing point should be four times -1.86° C. This is probably not quite true but the difference is so slight that it may be ignored here. It will thus be seen that with a solution in which there is a gram molecule to a liter of water of a solute that stays in solution below -29.76° C., there would be as much water unfrozen at -14.88° C., as there would be at -29.76° C., if the molar concentration is only half as great.

Maximow concludes that the protective action of the material he used was greater than could be explained by the depression of the freezing point of the sap. He may not have considered the above facts. Another fact should be considered. With the plants Maximow used, possibly a large percentage of the sap solute crystallized out at temperatures considerably above the minimum temperatures that he used. Maximow used as a protective substance a solution of equal density with the plant sap and with a low eutectic point, then at a temperature of say -10° C., it would be considerably more dense than the plant sap from which crystallization had taken place. While theoretically the protective action of increased density should be greater with plants that kill at a rather low temperature like winter buds and wood, we have found no way to demonstrate such protective action with such tissue. The only means of increasing the sap density of such tissue that suggested itself to us was by applying fertilizers like potassium chloride to the soil and this method has not had that effect.

From the experience of Maximow and experience here with cabbage and other plants where the salts in solution have increased the resistance to cold as much as it was increased by organic compounds, and in some cases more, it seems that one is safe in concluding that killing from cold is more likely a mechanical injury due to the withdrawal of water from the protoplasmic membrane than an injury resulting from the precipitation of proteids as suggested by Gorke, since an increase in the percentage of any mineral salt should tend to hasten the precipitation of proteids rather than reduce it.

It seemed that one good test of the theory of Gorke[1] would be to find whether or not a lower temperature is required to precipitate proteids from the sap of twigs as they increase in hardiness during

[1]Landw. Versuchs. Vol. 65, p. 149, 1906. (Bibl. No. 47).

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early winter. It is known that from the time the leaves fall for at least one or one and one-half months, the cortex as well as the winter buds and the sap wood of deciduous trees (at least of peaches) continue to increase in hardiness. This Station made an effort to determine whether this increase in hardiness might not be due to such a change in the composition of proteids that they would tend to remain in solution at lower temperatures. Consequently twigs were gathered on the dates shown in the following table, beginning before leaf fall. Large quantities of cortex sap were expressed. After pouring back and forth to insure a uniform solution, one-half of this sap was frozen at the temperatures shown in the table, a temperature that would at that time kill the cortex and practically all other tissue of the twig. The other half was kept in an ice box until the freezing of the other sap was complete, then both were taken to the Department of Agricultural Chemistry for proteid analysis, the frozen sap being thawed on the filter except when otherwise stated in the tables. The analysis was by the following plan, kindly furnished by the Department of Agricultural Chemistry:

KJELDAHL-GUMMING METHOD FOR ESTIMATION OF NITROGEN.

"Weigh out accurately 4-6 grams of the sap by difference, place in 500 c. c. Kjeldahl flask, add 0.7 gram of mercury and 25 c. c. of sulphuric acid, heat for a few minutes until frothing ceases, add 7 grams of potassium sulphate and digest over a flame until clear, cool; wash down the neck of the flask with distilled water, heat again one hour until water is expelled. Cool and dilute with water until flask is 2 / 3 full, add a piece of zinc and a piece of paraffin and 80 c. c. of alkaline solution containing 500 grams potassium sulphide and 18 kilos sodium hydroxide in 40 liters of the solution. Distil into standard hydrochloric acid and titrate back with standard ammonium hydroxide, using cochineal as indicator". The factor 6.25 was used in reducing nitrogen reading to proteid readings.

Twigs of apple, peach, pear and plum were used with results as shown in the following table:

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TABLE 12. SHOWING PERCENTAGE OF PROTEIDS IN FROZEN AND UNFROZEN SAP FROM TISSUES OF APPLE, PEAR, PEACH AND PLUM.

Kind of Sap	Date	Temperature	Average per cent proteids. Sap unfrozen	Average per cent proteids. Sap frozen
Wealthy apple twigs.....	Sept. 4,'11	−17	0.397	0.435
Wealthy apple twigs.....	Oct. 2,'11	− 9.5	0.556	0.544
Elberta peach twigs.....	Sept. 4,'11	−17	1.488	1.894
Elberta peach twigs.....	Oct. 2,'11	− 9.5	2.644	2.550
Elberta peach twigs.....	Oct. 16,'11	− 9.5	2.550	2.531
Elberta peach roots.....	Oct. 16,'11	− 9.5	1.563	1.563
Elberta peach twigs.....	Nov. 30,'11	− 9	2.050	2.100
Chabot plum twigs.....	Sept. 4,'11	−17	0.651	0.694
Chabot plum twigs.....	Oct. 2,'11	− 9.5	0.819	0.800
Kieffer pear twigs.....	Sept. 4,'11	−17	0.144	0.031
Kieffer pear twigs.....	Oct. 2,'11	− 9.5	0.406	0.375
Kieffer pear twigs.....	Oct. 16,'11	− 9.5	0.288	0.288
Kieffer pear roots.....	Oct. 16,'11	− 9.5	0.181	0.206
Kieffer pear twigs.....	Nov. 30,'11	− 9	0.350	0.375

In no case does there seem to be any conclusive indications that proteids were precipitated by temperatures low enough to kill the plant tissue. Since there was no precipitation from the sap of tender twigs in early autumn, sap was not taken from twigs later in the season.

Cabbage and other succulent plants grown in the greenhouse, some well watered, some under dry conditions and others grown out of doors, also well watered but under conditions where they would withstand lower temperatures than would those grown in the greenhouse, had each an abundance of sap expressed, half of it being frozen to a temperature that would kill those well watered in the greenhouse, but would not kill those grown out of doors. Samples of each of these frozen and unfrozen were analyzed for proteids, with the results shown in the following table:

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TABLE 13. SHOWING PERCENTAGE OF PROTEIDS IN FROZEN AND UNFROZEN SAP FROM SUCCULENT PLANTS.

Kind of Sap	Date	Temperature	Average per cent proteids. Sap frozen.	Average per cent proteids. Sap unfrozen.
Cabbage greenhouse, dry soil	Apr. 8,'12	-3	0.975	1.069
Cabbage, greenhouse	Apr. 8,'12	-3	0.781	0.838
Cabbage, out-of-doors	Apr. 8,'12	-3	1.406	1.438
Cabbage sap, outside	Nov. 28,'11	-5	1.675	1.856
Cabbage, inside dry soil	Oct. 28,'11	-5	1.144	1.281
Cabbage, inside	Oct. 28,'11	-5	0.919	0.956
Tomato sap, greenhouse	Dec. 1,'11	-3.7	0.594	0.438
Tomato sap, greenhouse, dry soil	Dec. 1,'11	-3.7	1.313	1.444
Tomato sap, outside	Dec. 1,'11	-3.7	0.700	0.713
Pea sap, inside	Apr. 27,'12	-5	2.069	2.413
Pea sap, outside	Apr. 27,'12	-5	3.088	1.625
Lettuce, sap inside	Apr. 27,'12	-5	0.519	0.613
Lettuce sap, outside	Apr. 27,'12	-5	0.269	0.263
Kale, greenhouse	Dec. 16,'11	-6.2	1.744	1.831
Kale, outside	Dec. 16,'11	-6.2	1.963	1.994

In case of cabbage, kale, lettuce and peas there is some evidence of precipitation in case of the more tender greenhouse plants, but it is possible that the differences are within the range of experimental error.

In March, 1913, apple roots that had been growing in the greenhouse for sixty days had sap expressed, half of it frozen to a temperature that would kill them, the other half not frozen. At the same time sap was taken from roots that had been kept in cold storage at a temperature seldom varying from 32°F., with the following results:

TABLE 14. SHOWING PERCENTAGE OF PROTEIDS IN FROZEN AND UNFROZEN SAP FROM APPLE ROOTS.

Material	Treatment	Date	Percentage Nitrogen
Apple stock, cold storage	Not frozen..	Mar. 14,'13	0.325
Apple stock, cold storage	Frozen.....	Mar. 14,'13	0.325
Apple stock, greenhouse	Not frozen..	Mar. 14,'13	0.592
Apple stock, greenhouse	Frozen.....	Mar. 14,'13	0.548

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In case of the greenhouse grown apple stock there seems to be slight evidence that proteids were precipitated by the low temperature. However, the difference is probably within the range of error. In these cases great care was taken to prevent any change in the soluble proteid content of the sap before analysis began. The unfrozen sap was immediately started for analysis, after being expressed. In all cases the frozen sap was thawed on filter paper so that little change could take place after the thawing.

From the above tables, we are safe in concluding that in the case of the cortex of twigs from fruit trees in autumn or early winter, precipitation of proteids plays no part in the killing since temperatures that will kill all the cortex will precipitate no proteids. In the case of some succulent growing plants there seems some evidence that a killing temperature will precipitate a slight percentage of the soluble proteids present, but since increasing the amount of salts present that are supposed to precipitate the proteids increases instead of decreasing the resistance to low temperature, we are probably safe in concluding that precipitation of proteids does not explain freezing to death.

OTHER FEATURES THAT INFLUENCE THE FREEZING TO DEATH OF PLANTS.

Rate of Thawing. The effect of the rate of thawing on the killing from cold has been discussed previously with reference to the works of Müller-Thurgau,[1] Molisch,[2] and Sachs.[3] However, it is not out of place to discuss it briefly as one influence to be dealt with in the killing of certain plants from cold. As mentioned before, Müller-Thurgau and Molisch found only mature fruits and leaves of Agava Americana to be affected by the rate of thawing. Practically all plants used in freezing experiments to be reported in this paper have been tested with reference to the effect of rapid thawing, particularly because of the effect such thawing would have on the results with other experiments. The following table gives the results with peach fruit buds that were thawed slowly and rapidly:

[1]Landw. Jahrb. Vol. 15, p. 453, 1886. (Bibl. No. 78).
[2]Untersuchung über das Erfrieren, etc. Book, 1897. (Bibl. No. 75).
[3]Ber. u. d. Ver. d. Kon. Sachs. Gesell. d. Wiss. zu. Leipzig, 1860, Vol. 12, pp. 1-50. (Bibl. No. 94).

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TABLE 15. SHOWING THE EFFECT OF SLOW AND RAPID THAWING ON THE AMOUNT OF KILLING OF PEACH FRUIT BUDS.

Variety	Treatment	Date	Temperature	Number of Buds	Percentage Killed
Hills Chili	Thawed rapidly	Jan. 21,'11	-21	178	40
Hills Chili	Thawed slowly	Jan. 21,'11	-21	200	51.1
Hills Chili	Thawed rapidly	Jan. 22,'11	-20.5	100	7.0
Hills Chili	Thawed slowly	Jan. 22,'11	-20.5	100	23.0
Lewis	Thawed rapidly	Feb. 9,'11	-20.2	100	7.0
Lewis	Thawed slowly	Feb. 9,'11	-20.2	100	20.0
Elberta	Thawed rapidly	Dec. 8,'11	-16	110	20.9
Elberta	Thawed slowly	Dec. 8,'11	-16	100	8.0
Elberta	Thawed rapidly	Dec. 13,'11	-16	75	94.6
Elberta	Thawed slowly	Dec. 13,'11	-16	80	100.0
Rice's Seedling	Thawed rapidly	Mar. 24,'13	-16	129	72.2
Rice's Seedling	Thawed slowly	Mar. 24,'13	-16	130	84
Elberta	Thawed rapidly	Mar. 24,'13	-16	95	95.8
Elberta	Thawed slowly	Mar. 24,'13	-16	83	90.4
Average, Thawed rapidly					38.93
Average, Thawed slowly					53.61

The buds were thawed slowly by simply lifting the inner cylinder to which they were attached and seeing that the temperature was raised to four or five degrees above that to which they were lowered. In no case was it possible that the temperature could have gone back to the minimum temperature recorded, and usually twelve hours, sometimes slightly more, were required for the thawing.

Enough buds were frozen to justify a positive conclusion that rapid thawing does not influence the amount of killing.

The following table gives the results of slow and rapid thawing of young fruits:

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TABLE 16. SHOWING THE EFFECT OF THE RATE OF THAWING ON FREEZING TO DEATH OF YOUNG FRUIT.

Kind of Fruit	Age of Fruit in days	Date	Manner of Thawing	Temperature	Number of Fruits	Percentage Killed
Lewis peach.....	30	May 17,'13	Rapidly in room.....	−4	23	43.5
Lewis peach.....	30	May 17,'13	Slowly in freezer....	−4	24	25.0
Early Bernard.... peach.....	35	May 21,'13	Rapidly in room.....	−4	34	61.8
Early Bernard peach.....	35	May 21,'13	Slowly in freezer....	−4	34	91.2
Elberta Seedling peach.....	38	May 24,'13	Rapidly in room.....	−4	21	61.9
Elberta Seedling peach.....	38	May 24,'13	Slowly in room.....	−4	30	30.0
Elberta Seedling peach.....	38	May 24,'13	Rapidly, fanned....	−4	25	44.0
Carman peach.. dia. 1 in.....	44	May 31,'13	Rapidly in room.....	−4	20	40.0
Carman peach dia. 1 in.....	44	May 31,'13	Rapidly in sun.....	−4	20	45.0
Carman peach dia. 1 in.....	44	May 31,'13	Rapidly, fanned....	−4	20	45.0
Carman peach dia. 1 in.....	44	May 31,'13	Slowly in freezer.....	−4	20	45.0
Waddell peach dia. 1¼ in.....	52	June 6,'13	Rapidly in room.....	−4.5	22	81.9
Waddell peach dia. 1¼ in.....	52	June 6,'13	Slowly in freezer....	−4.5	22	70.0
Waddell peach dia. 1¼ in.....	52	June 6,'13	Rapidly, fanned....	−4.5	22	80.0
Seedling peach....	..	June 11',13	Rapidly in room.....	−4.5	15	46.7
Seedling peach....	..	June 11,'13	Slowly in freezer....	−4.5	16	25.0
Jonathan apple....	23	May 17,'13	Rapidly in room.....	−4	19	68.4
Jonathan apple....	23	May 17,'13	Slowly in freezer....	−4	15	60.0
Jonathan apple....	30	May 24,'13	Rapidly in room.....	−4	23	56.5
Jonathan apple....	30	May 24,'13	Slowly in freezer....	−4	31	80.7
Jonathan apple....	30	May 24,'13	Rapidly, fanned....	−4	19	78.9
Salome apple.....	..	June 11,'13	Rapidly in room.....	−4	15	46.7
Salome apple.....	..	June 11,'13	Slowly in freezer....	−4	15	33.3
Dyehouse cherry..	..	May 17,'13	Rapidly in room.....	−4	15	66.7
Dyehouse cherry..	..	May 17,'13	Slowly in freezer....	−4	18	16.6
Dyehouse cherry..	..	May 21,'13	Rapidly in room.....	−4	38	39.5
Dyehouse cherry..	..	May 21,'13	Slowly in freezer....	−4	47	80.9
Average, Rapidly in room.....						55.8
Average, Slowly in freezer.....						50.7

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The fruits were thawed slowly by removing some of the salt and ice from the freezer and if necessary lifting the lid to the freezer so the temperature inside would immediately cool to about one degree centigrade above that to which the fruits were run. This was done in order to be certain that the slowly thawing fruits did not suffer greater killing by being kept at the minimum temperature longer than the rapidly thawed fruits. Approximately three hours were required for succulent plants and fruits to thaw in this way. It is apparently certain that the rate of thawing has little to do with the amount of killing of young peaches and apples. Not enough cherries were tried to justify conclusions.

It will be seen in the table that some of the fruits were fanned in thawing. These fruits were taken in the frozen condition from the freezer and put at once in the current from an electric fan to see if rapid evaporation during thawing and shortly afterward influences the killing. The results indicate that it does not.

Results at this station from freezing ripe winter apples agree with those of Müller-Thurgau and Molisch that unless the temperature goes too low, slow thawing will greatly reduce the amount of killing. With the very young fruits, however, there is no indication that the rate of thawing influences the killing.

The following table gives the results of freezing apples in early July that ripen in September; pears that ripen in August and September, ripe Yellow Swan peaches and Elberta peaches that ripen about August 15th in Columbia, and Krummel October peaches which ripen in September in Columbia:

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TABLE 16. SHOWING RELATIVE EFFECTS OF SLOW AND RAPID THAWING OF FROZEN GREEN FRUIT OF APPLE, PEAR, PEACH AND PLUM.

Material	Date	Temperature	Number of Fruits	Percentage of Fruits Showing Injury	Remarks
Jonathan apples, 1½ in. in diameter (Thawed rapidly)....	July 11,'13	−5	10	80	Seeds uninjured
Jonathan apples, 1½ in. in diameter (Thawed slowly).....	July 11,'13	−5	10	100	Seeds uninjured
Jonathan apples, half grown (Thawed rapidly)....	July 14,'13	−5	6	100	Slight injury
Jonathan apples, half grown (Thawed slowly).....	July 14,'13	−5	6	100	Slight injury
Jonathan apples, (Thawed rapidly)....	July 17,'13	−5	5	100	Slight injury
Jonathan apples, (Thawed slowly).....	July 17,'13	−5	5	100	Slight injury
Blenheim apples, ripe (Thawed rapidly)....	July 14,'13	−5	4	100	Slight injury
Blenheim apples, ripe (Thawed slowly).....	July 14,'13	−5	4	100	Slight injury
Duchess pear, 1½ in. in diameter (Thawed rapidly)....	July 11,'13	−5	10	70	Seeds of injured fruit dead
Duchess pear, 1½ in. in diameter.... (Thawed slowly)	July 11,'13	−5	10	60	Seeds of injured fruit dead
Tyson pear, half-grown (Thawed rapidly)....	July 14,'13	−5	4	100	Slight injury
Tyson pear, half-grown (Thawed slowly).....	July 14,'13	−5	4	100	Slight injury
Duchess pear, green (Thawed rapidly)....	July 17,'13	−5	5	100	Slight injury
Duchess pear, green (Thawed slowly).....	July 17,'13	−5	5	100	Slight injury
Krummel October peach (Thawed rapidly)....	July 12,'13	−5	10	100	Seeds of injured fruit killed
Krummel October peach (Thawed slowly).....	July 12,'13	−5	10	100	Flesh thawed in freezer little less injured

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Material	Date	Temperature	Number of Fruits	Percentage of Fruits Showing Injury	Remarks
Elberta peach, green (Thawed rapidly)....	July 14,'13	-5	5	100	Slight injury
Elberta peach, green (Thawed slowly).....	July 14,'13	-5	5	100	Slight injury
Yellow Swan peach, ripe (Thawed rapidly)....	July 14,'13	-5	4	100	Slight injury
Yellow Swan peach, ripe (Thawed slowly).....	July 14,'13	-5	4	100	Slight injury
Elberta peach, green (Thawed rapidly)....	July 17,'13	-5	5	100	Injury slight
Elberta peach, green (Thawed slowly).....	July 17,'13	-5	5	100	Injury slight
Graves peach, ripe (Thawed rapidly)....	July 17,'13	-5	5	100	Injury slight
Graves peach, ripe (Thawed slowly) ...	July 17,'13	-5	5	100	Injury slight
Wild Goose plum, green (Thawed rapidly)....	July 14,'13	-5	5	80	Injury rather severe
Wild Goose plum, green (Thawed slowly).....	July 14,'13	-5	5	100	Injury rather severe
Wild Goose plum, ripe (Thawed rapidly)....	July 14,'13	-5	5	100	Injury rather severe
Wild Goose plum, ripe (Thawed slowly)	July 14,'13	-5	5	100	Injury rather severe
Average, green fruits rapidly thawed				93.6	
Average, green fruits slowly thawed				96.4	

Those fruits that were thawed slowly were thawed in the same manner as the young fruits in the previous table. They were thawed as slowly as it was possible to thaw them. Ice without salt was packed over the freezer and left over night. For those frozen July 14th, the temperature at 5:05 P. M. was -4.5° C; at 5:30 P. M., -3.5° C; at 5:45 P. M., -3° C, and at 6:30 A. M. the next day it was 9.5° C. The temperature rise for the other freezings was approximately the same. The ice was all out of the fruits thawed rapidly in a warm

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room in a few minutes. In no case was there any evidence that the rate of thawing affected the amount of injury to the unripe fruit, or even to the partially ripe Yellow Swan peaches. It seems probable that slow thawing affects the amount of injury to fruits only in case of ripe apples and pears.

The following table gives the results of slow and rapid thawing of some garden plants:

TABLE. 17. SHOWING THE EFFECT OF SLOW AND RAPID THAWING ON LEAVES AND STEMS OF GROWING PLANTS.

Material	Manner of Thawing	Date	Temperature	Number of Leaves	Percentage all Killed	Percentage Total Surface Killed
Cowpeas.....	Rapidly in room.	May 28,'13	-3.8	12	25	
Cowpeas.....	Slowly in freezer	May 28,'13	-3.8	26	100	
Cowpeas.....	Rapidly in sun..	May 28,'13	-3.8	25	100	
Cowpeas.....	Rapidly in room.	June 14,'13	-4	3	100	100
Cowpeas.....	Slowly in freezer.	June 14,'13	-4	3	..	90
Lettuce.....	Rapidly in room.	May 7,'13	-4	27	22.2	37
Lettuce.....	Slowly in freezer.	May 7,'13	-4	20	5.0	13
Lettuce.....	Rapidly in room.	May 13,'13	-4	4	...	90
Lettuce.....	Slowly in freezer.	May 13,'13	-4	3	..	50
Lettuce (Black Seeded Simpson)	Rapidly in room.	June 12,'13	-4	9	66.6	92
Lettuce (Black Seeded Simpson)	Slowly in freezer.	June 12,'13	-4	11	36.6	50
Lettuce (Black Seeded Simpson)	Rapidly in room.	June 12,'13	-4	10	90.0	97.5
Lettuce (Black Seeded Simpson)	Slowly in freezer.	June 12,'13	-4	10	90.0	97.5
Kale.....	Rapidly in room.	May 15,'13	-5	6	16.6	20.8
Kale.....	Slowly in freezer.	May 15,'13	-5	5	0.0	0.0
Kale.....	Rapidly in room.	June 28,'13	-3.5	5	100.0	100.0
Kale.....	Slowly in freezer.	June 28,'13	-3.5	5	100.0	100.0
Kale,.....	Rapidly, fanned.	June 28,'13	-3.5	5	100.0	100.0
Kale.....	Rapidly in room.	July 3,'13	-3.5	5	80.0	95.0
Kale.....	Slowly in freezer.	July 3,'13	-3.5	5	80.0	95.0
Cabbage.....	Rapidly in room.	May 15,'13	-5	4	100.0	100.0
Cabbage.....	Slowly in freezer.	May 15,'13	-5	4	100.0	100.0
Red Cabbage.....	Rapidly in room.	May 15,'13	-5	5	20.0	20.0
Red Cabbage.....	Slowly in freezer	May 15,'13	-5	9	33.3	33.3
Cabbage, Flat Dutch.....	Rapidly in room.	June 12,'13	-4	20	100.0	100.0
Cabbage, Flat Dutch.....	Slowly in room..	June 12,'13	-4	10	100.0	100.0
Cabbage.....	Rapidly in room.	June 28,'13	-3.5	5	40.0	85.0
Cabbage.....	Slowly in freezer.	June 28,'13	-3.5	5	40.0	85.0
Cabbage.....	Rapidly, fanned.	June 28,'13	-3.5	5	40.0	85.0

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Material	Manner of Thawing	Date	Temperature	Number of Leaves	Percentage all Killed	Percentage Total Surface Killed
Cabbage, Red Rock.....	Rapidly in room.	June 30,'13	−4	5	40.0	65.0
Cabbage, Red Rock.....	Slowly in freezer.	June 30,'13	−4	5	60.0	65.0
Cabbage, Red Rock.....	Rapidly, fanned.	June 30,'13	−4	5	60.0	70.0
Tomatoes.....	Rapidly in room.	June 28,'13	−3.5	26	100.0	100.0
Tomatoes.....	Slowly in freezer.	June 28,'13	−3.5	25	100.0	100.0
Tomatoes.....	Rapidly, fanned.	June 28,'13	−3.5	25	100.0	100.0
Elberta peach leaves....	Rapidly.....	July 16,'13	−6	9	44.4	58.9
Elberta peach leaves....	Slowly.....	July 16,'13	−6	8	0.0	25.0
Elberta peach leaves....	Rapidly.....	July 17,'13	−5	57	0.0	17.9
Elberta peach leaves....	Slowly.....	July 17,'13	−5	58	0.0	18.9
Late Duchess apple leaves.....	Rapidly.....	July 16,'13	−6	22	0.0	28.4
Late Duchess apple leaves.....	Slowly.....	July 16,'13	−6	19	0.0	19.7
Late Duchess apple leaves.....	Rapidly.....	July 17,'13	−5	51	0.0	27.4
Late Duchess apple leaves.....	Slowly	July 17,'13	−5	55	0.0	26.3
Average, Leaves of succulent plants, thawed slowly.....						68.4
Average, Leaves of succulent plants, thawed rapidly.....						76.3
Average, Same, excluding lettuce, thawed slowly.....						74.4
Average, Same, excluding lettuce, thawed rapidly.....						76.2

In none of these plants, except lettuce, does there seem to be any difference in the amount of killing on account of the rate of thawing. Lettuce, however, seems to have the amount of injury reduced by slow thawing. Even with lettuce, however, a slight reduction in temperature, certainly not more than one degree, would offset the effect of slow thawing.

Effect of Wilted Condition on Killing Temperature. The condition of plant tissue at the time of freezing with reference to turgidity seems to have something to do with the killing. In all freezings care has been taken to have all of the plants kept in an equally turgid condition, or as nearly so as it was possible to maintain, though to find to what extent slight difference in turgidity might alter results, a considerable number of plants were frozen, some turgid and some wilted. The following table gives a list of freezings and the results:

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TABLE 18. SHOWING EFFECT OF WILTING OF PLANT TISSUE ON ITS RESISTANCE TO COLD (FRUITS).

Material	Date	Temperature	Number of Fruits	Percentage Killed	Percentage Weight Lost
PEACHES					
Elberta Seedling, turgid	May 24,'13	−4	21	61.9	
Elberta Seedling, wilted on twig	May 24,'13	−4	29	17.2	
Elberta Seedling, wilted 3½ hrs. detached	May 24,'13	−4	29	24.2	27.6
Lewis, fresh	May 19,'13	−4	23	43.5	
Lewis, wilted 6 hrs. on twig	May 19,'13	−4	22	45.5	
Early Bernard, turgid	May 21,'13	−4	34	61.8	
Early Bernard, wilted 5 hrs. on twig	May 21,'13	−4	53	69.8	4.0
APPLES					
Jonathan, turgid	May 24,'13	−4	23	56.5	
Jonathan, wilted on twig	May 24,'13	−4	29	83.8	2.0
Jonathan, wilted detached	May 24,'13	−4	26	65.4	
Jonathan, turgid	May 19,'13	−4	19	68.4	
Jonathan, wilted on twig 6 hrs.	May 19,'13	−4	37	86.5	
Salome, turgid	June 12,'13	−4.5	15	46.7	
Salome, wilted on twig	June 12,'13	−4.5	12	50.0	
CHERRIES					
Dyehouse, turgid	May 21,'13	−4	38	39.5	
Dyehouse, wilted 5 hrs. on twig	May 21,'13	−4	56	21.4	3.4
Average percentage of fruits killed, fresh				54.0	
Average percentage of fruits killed, wilted on twig				53.5	

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TABLE 18a. SHOWING EFFECT OF WILTING OF PLANT TISSUE ON ITS RESISTANCE TO COLD. (SUCCULENT PLANTS.)

Material	Date	Temperature	Number of Leaves	Percentage all Killed	Percentage Total Surface Killed	Percentage Weight Lost
Tomato, turgid	Aug. 10,'12	-3	2		80.0	
Tomato, wet and turgid	Aug. 10,'12	-3	2		90.0	
Tomato, wilted	Aug. 10,'12	-3	2		85.0	
Tomato, turgid	Aug 11,'12	-2	2		20.0	
Tomato, wet and turgid	Aug. 11,'12	-2	2		40.0	
Tomato, wilted	Aug. 11,'12	-2	2		30	
Tomato, turgid	June 25,'13	-2.5	32	50.0	72.6	
Tomato, wet and turgid	June 25,'13	-2.5	32	69.2	88.4	
Tomato, wilted	June 25,'13	-2.5	37	43.2	62.8	15.6
Tomato, turgid	June 30,'13	-2.5	35	....	97.5	
Tomato, wilted	June 30,'13	-2.5	24	....	58.0	28.0
Kale, turgid	July 3,'13	-3.5	5	80.0	95.0	
Kale, wilted	July 3,'13	-3.5	5	60.0	85.0	35.4
Red Rock Cabbage, turgid	June 30,'13	-4	5		65.0	
Red Rock Cabbage, wilted	June 30,'13	-4	4		43.9	16.8
Lima bean, turgid	June 22,'12	-2	5		15.0	
Lima bean, wilted	June 22,'12	-2	5		75.0	
Lettuce, turgid	July 25,'12	-2	5		0.0	
Lettuce, wet and turgid	July 25,'12	-2	5		50.0	
Lettuce, wilted	July 25,'12	-2	5		0.0	
Lettuce, turgid	July 27,'12	-2	5		0.0	
Lettuce, wet and turgid	July 27,'12	-2	5		30.0	
Lettuce, wilted	July 27,'12	-2	5		60.0	
Lettuce, turgid	July 30,'12	-2	5		25.0	
Lettuce, wet and turgid	July 30,'12	-2	5		25.0	
Lettuce, wilted	July 30,'12	-2	5		35.0	
Black Seeded Simpson lettuce, turgid	June 12,'13	-4	9	92.5	66.6	
Black Seeded Simpson lettuce, wilted	June 12,'13	-4	9	33.3	55.5	23.4
Black Seeded Simpson lettuce, turgid	June 13,'13	-4.5	12	75.0	83.0	
Black Seeded Simpson lettuce, wilted	June 13,'13	-4.5	14	57.0	62.0	21.8
Turnip, turgid	July 25,'12	-2	4		0.0	
Turnip, wet and turgid	July 25,'12	-2	4	....	30.0	....
Turnip, wilted	July 25,'12	-2	4	....	30.0	....
Turnip, turgid	July 27,'12	-2	4	....	40.0	....
Turnip, wet and turgid	July 27,'12	-2	4	....	10.0	....
Turnip, wilted	July 27,'12	-2	4	....	10.0	....
Red Clover, turgid	July 22,'12	-2	4	....	17.0	....
Red Clover, wilted	July 22,'12	-2	4	....	34.0	....
Red Clover, turgid	July 24,'12	-3	4	....	100.0	....
Red Clover, wilted	July 24,'12	-3	4	....	66.0	....
Rose Geranium, turgid	July 22,'12	-2	4	....	97.0	....
Rose Geranium, wilted	July 22,'12	-2	4	....	60.0	....
Rose Geranium, turgid	July 24,'12	-3	4	....	100.0	....

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Material	Date	Temperature	Number of Leaves	Percentage all Killed	Percentage Total Surface Killed	Percentage Weight Lost
Rose Geranium, wet and turgid	July 24,'12	-3	4	....	100.0	....
Rose Geranium, wilted	July 24,'12	-3	4	....	100.0	....
Common Geranium, turgid	July 24,'12	-3	4	....	95.0	....
Common Geranium, wet and turgid	July 24,'12	-3	4	....	90.0	....
Common Geranium, wilted	July 24,'12	-3	4	....	100.0	....
Common Geranium, turgid	July 31,'12	-2	4	....	100.0	....
Common Geranium, wet and turgid	July 31,'12	-2	4	....	80.0	....
Common Geranium, wilted	July 31,'12	-2	4	....	100.0	....
Morning-glory, turgid	July 31,'12	-2	3	....	100.0	....
Morning-glory, wet and turgid	July 31,'12	-2	3	....	100.0	....
Morning-glory, wilted	July 31,'12	-2	3	....	0.0	....
Morning-glory, turgid	Aug. 3,'12	-2	4	....	15.0	....
Morning-glory, wet and turgid	Aug. 3,'12	-2	4	....	100.0	....
Morning-glory, wilted	Aug. 3,'12	-2	4	....	15.0	....
Coleus, turgid	Sept. 3,'12	-2.5	4	....	25.0	....
Coleus, wet and turgid	Sept. 3,'12	-2.5	4	....	50.0	....
Coleus, wilted	Sept. 3,'12	-2.5	4	....	100.0	....
Fern, turgid	Sept. 6,'12	-2	5	....	0.0	....
Fern, wet and turgid	Sept. 6,'12	-2	5	....	15.0	....
Fern, wilted	Sept. 6,'12	-2	5	....	60.0	....
Fern, turgid	Sept. 7,'12	-2	5	....	5.0	....
Fern, wet and turgid	Sept. 7,'12	-2	5	....	7.0	....
Fern, wilted	Sept. 7,'12	-2	5	....	20.0	....
Fern, turgid	Sept. 9,'12	-2	5	....	40.0	....
Fern, wet and turgid	Sept. 9,'12	-2	5	....	55.0	....
Fern, wilted	Sept. 9,'12	-2	5	....	40.0	....
Mulberry, turgid	Sept. 9,'12	-2	5	....	5.0	....
Mulberry, wet and turgid	Sept. 9,'12	-2	5	....	0.0	....
Mulberry, wilted	Sept. 9,'12	-2	5	....	0.0	....
Average percentage surface killed, leaves of succulent plants, turgid					50.3	
Average percentage surface killed, leaves of succulent plants, wilted					51.4	

On January 22, 1913, apple roots from one-year-old seedling stock were frozen to -9° C in 3¼ hours:

(a) Two kept in water 18 hours.

All were injured. The crown cuts slightly in cambium; and the second and third in cambium and cortex severely.

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(b) Two roots kept in saw dust.

The crown cuts were very slightly injured; the second and third were more severely than the crown cuts, but less severely than the second and third cuts of (a).

(c) Two dried roots—allowed to dry on desk eighteen hours.

Crown cuts and second cuts entirely uninjured. Very slight injury in cambium region of third cut.

There is some indication that rapid wilting in case of some plants reduces the injury from freezing. However, in the case of most plants the difference is rather slight and with many plants there is no apparent difference. In all cases the wilting was sufficient to give the tissue a limp appearance. It seems rather certain then that wilting so slight that it can not be easily detected by the appearance or feel of the tissue could not influence the results in freezing such tissue as our plants with roots or stem bases in solutions. In case of plants with the surface wet, listed in the table as wet and turgid, there seems strong evidence that the killing from freezing is worse.

Long continued partial withholding of water is different. The rate of growth, sap density and possibly the resistance of the protoplasm to the results of low temperature are increased. There is no apparent reason to expect this of rapid wilting. It would temporarily increase the sap density by reducing the amount of water in the cell. It probably does not, however, increase the amount of material in the cell to hold water. Plants growing with insufficient water supply are generally more resistant to cold (See tables IV, V, and VI). We have also some results of this effect of continued partial withholding of water on dormant peach buds. On November 20, 1910, typical branches of peach trees were girdled, a ring being cut nearly through the sap wood, and another limb was cut off and set up in the tree. This was done with several trees. The following table gives the percentage of buds killed when the temperature went to -8° F., on January 3, 1911:

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TABLE 19. SHOWING EFFECT OF SLOW DRYING OUT ON HARDINESS OF PEACH BUDS IN WINTER.

Variety	Buds From	Date	Number of Buds	Percentage Killed
Barnes.....	Girdled branch.....	Jan. 14,'11	321	19.3
Barnes.....	Cut off branch.....	Jan. 14,'11	275	29.5
Barnes.....	Check, normal branch.....	Jan. 14,'11	325	86.0
Connolly....	Girdled branch.....	Jan. 14,'11	198	68.7
Connolly....	Cut off branch.....	Jan. 14,'11	281	68.0
Connolly....	Check, normal branch.....	Jan. 14,'11	217	89.9
Hills Chili...	Girdled branch.....	Jan. 14,'11	501	11.4
Hills Chili...	Cut off branch.....	Jan. 14,'11	351	18.0
Hills Chili...	Check, normal branch.....	Jan. 14,'11	465	45.2
Average, Girdled branch.....				33.1
Average, Cut off branch.....				38.5
Average, Check, normal branch.....				73.7

It is plain that this slow loss of water in case of winter resting fruit buds has increased the resistance of the tissue to low temperature.

Rate of Freezing. Pfeffer[1] makes the following statement with reference to the rate of freezing: "Resistant plants withstand rapid and slow cooling equally well, and it is doubtful whether a rapid fall of temperature is more injurious to plants killed by freezing than is gradual cooling. That the injury is not due to the sudden formation of ice after sub-cooling is shown by the fact that a peeled potato is killed by freezing, although no sub-cooling occurs and the ice forms gradually at -1° C., the freezing point of the sap." However, lately Winkler[2], working with Pfeffer, finds that with winter twigs that on cooling rapidly to -22° C. will be killed, if they are kept for three days at -16° C., two days at -18° C., three days at -20° C., two days at -22° C., three days at -25° C., and twelve hours at -30° to -32° C., they were not all killed.

The rate of temperature fall is very important indeed, especially in case of winter buds. In fact apple buds can be frozen in a chamber surrounded by salt and ice rapidly enough that practically all of them will be killed at a temperature of zero F., or slightly below, while it is well known that they may go through a temperature of

[1]Phys. of Plants. Eng. Trans. by Ewart, Vol. 2, p. 235. (Bibl. No. 88).
[2]Jahrb. f. Wiss. Bot. Vol. 52, 1913, pp. 467-506. (Bibl. No. 121).

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20° F. to 30° F. below zero with but slight injury where the temperature fall is not so rapid. No fruit buds have been found on the Experiment Station grounds that can not be killed by a temperature secured by salt and ice when in the most dormant state, by very rapid fall. The following is a list of the freezings in our laboratory, with the results:

TABLE 20. SHOWING EFFECT OF SLOW AND RAPID TEMPERATURE FALL ON FREEZING TO DEATH OF PLANT TISSUE.

Kind of Buds	Date	Number Buds	Percentage Killed	Number Buds	Percentage Killed
		Slowly to -19.5° C.		Rapidly to -17.7° C.	
Rice's Seedling peach	Jan. 25,'13	80	15	55	96.4
Yellow Swan peach	Jan. 25,'13	64	28	86	100.0
Early Richmond cherry	Jan. 25,'13	100	16	90	97.7
		Slowly to -20° C.		Rapidly to -19° C.	
Rice's Seedling peach	Feb. 15,'13	117	10.3	68	100.0
Yellow Swan peach	Feb. 15,'13	95	30.5	64	100.0
Early Richmond cherry	Feb. 15,'13	100	45.0	89	98.8
Dyehouse cherry	Feb. 15,'13	83	22.9	90	97.6
		Slowly to -18.5° C.		Rapidly to -12° C.	
Rice's Seedling peach	Mar. 15,'13	104	42.3	101	37.6
Elberta peach	Mar. 15,'13	104	97.7	86	61.6
Jonathan apple	Mar. 15,'13	35	34.3	20	70.0
Montmorency cherry	Mar. 15,'13	85	14.1	87	17.2
Lombard plum	Mar. 15,'13	112	12.9	99	47.3
		Slowly to -15.5° C.		Rapidly to -10° C.	
Hills Chili peach	Feb. 22,'13	63	17.3	49	10.2
Rice's Seedling peach	Feb. 22,'13	104	10.6	81	4.9
Early Richmond cherry	Feb. 22,'13	94	29.8	73	54.8
		Slowly to -18° C.		Rapidly to -13.5° C.	
Elberta peach	Mar. 8,'13	66	30.3	92	70.6
Hills Chili peach	Mar. 8,'13	120	20.8	71	76.0
Rice's Seedling peach	Mar. 8,'13	70	21.4	82	54.9
		Slowly to -18° C.		Rapidly to -13.5° C.	
Rice's Seedling peach	Mar. 22,'13	138	44.2	154	51.9
Elberta peach	Mar. 22,'13	100	88.0	85	92.9
Jonathan apple	Mar. 22,'13	34	64.7	33	75.7
Montmorency cherry	Mar. 22,'13	176	58.5	184	62.5
Chabot plum	Mar. 22,'13	236	78.3	183	86.8

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Kind of Buds	Date	Number Buds	Percentage Killed	Number Buds	Percentage Killed
		Slowly to -16.5° C.		Rapidly to -11.5° C.	
Rice's Seedling peach	Mar. 24,'13	145	57.0	158	53.2
Elberta peach	Mar. 24,'13	170	82.9	86	75.5
Jonathan apple	Mar. 24,'13	39	46.2	40	70.0
Montmorency cherry	Mar. 24,'13	166	76.5	189	59.2
Chabot plum	Mar. 24,'13	191	100.0	176	73.8
		Slowly to -16° C.		Rapidly to -12.5° C.	
Elberta peach	Nov. 3,'10	382	77.8	188	69.1
		Slowly to -16.5° C.		Rapidly to -16° C.	
Elberta peach	Dec. 7,'11	150	1.3	200	100.0
		Slowly to -16.2° C.		Rapidly to -16.5° C.	
Elberta peach	Dec. 7,'11	210	14.8	150	96.6

TABLE 20a. SHOWING EFFECT OF SLOW AND RAPID TEMPERATURE FALL ON FREEZING TO DEATH OF PLANT TISSUE.

Kind of Buds	Manner of Freezing	Date	Number Buds	Percentage Killed
Montmorency cherry	Slowly to -20° C.	Mar. 2,'12	163	3.0
Montmorency cherry	Rapidly to -20° C.	Feb. 29,'12	130	96.0
Early Richmond cherry	Slowly to -20° C.	Mar. 9,'12	297	5.0
Early Richmond cherry	Rapidly to -20° C.	Mar. 14,'12	263	98.0

TABLE 20b. SHOWING EFFECT OF SLOW AND RAPID TEMPERATURE FALL ON FREEZING TO DEATH OF PLANT TISSUE.

Kind of Buds	Date	Temperature	Time	Number Buds	Percentage Killed
Ben Davis apple	Jan. 18,'12	-20.5	2 hrs.	40	50.0
Ben Davis apple	Jan. 19,'12	-20.5	2 hrs.	47	36.2
Ben Davis apple	Feb. 2,'12	-20	1½ hrs.	60	0.0
Ben Davis apple	Feb. 3,'12	-20	2½ hrs.	82	2.44
Jonathan apple	Jan. 18,'12	-20.5	2 hrs.	35	48.6
Early Richmond cherry	Jan. 18,'12	-21	1½ hrs.	272	98.6
Early Richmond cherry	Jan. 19,'12	-20.5	2 hrs.	200	100.0
Montmorency cherry	Jan. 19,'12	-20.5	1¼ hrs.	178	87.0
Early Richmond cherry	Feb. 9,'12	-20.5	1½ hrs.	252	100.0
Montmorency cherry	Feb. 10,'12	-20.5	1¼ hrs.	195	94.8
Cherry	Feb. 15,'12	-20.5	12 hrs. (slowly)	132	38.0
	Rapidly, +20° C. to -17° C. in 45 minutes				
Jonathan apple	Feb. 20,'13			20	100.0
Vermont Beauty pear	Feb. 20,'13			25	100.0
Chabot plum	Feb. 20,'13			28	100.0

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TABLE 20c. SHOWING EFFECT OF SLOW AND RAPID TEMPERATURE FALL ON FREEZING TO DEATH OF PLANT TISSUE.

Material	Date	Time	Temperature	Results
Elberta peach twigs	Mar. 21,'13	7¼ hrs.	−18	Sap wood and pith only injured regions. Rice's Seedling showed the least injury. Others injured equally. Killing about the same for twigs whose temperature fell rapidly to −13.5° and those whose temperature fell slowly to −18°. (Buds injured worst in rapidly frozen ones).
Rice's Seedling peach twigs	Mar. 21,'13	7¼ hrs.	−18	
Jonathan apple twigs	Mar. 21,'13	7¼ hrs.	−18	
Chabot plum twigs	Mar. 21,'13	7¼ hrs.	−18	
Montmorency cherry twigs	Mar. 21,'13	7¼ hrs.	−18	
Elberta peach twigs	Mar. 21,'13	1¾ hrs.	−13.5	
Rice's Seedling peach twigs	Mar. 21,'13	1¾ hrs.	−13.5	
Jonathan apple twigs	Mar. 21,'13	1¾ hrs.	−13.5	
Chabot plum twigs	Mar. 21,'13	1¾ hrs.	−13.5	
Montmorency cherry twigs	Mar. 21,'13	1¾ hrs.	−13.5	
Elberta peach twigs	Mar. 22,'13	1 hr.	−11.5	Pith and sap wood injured.
Elberta peach twigs	Mar. 22,'13	7½ hrs.	−16.5	Pith and sap wood injured. Very slight browning in cortex; none in cambium.
Rice's Seedling peach twigs	Mar. 22,'13	1 hr.	−11.5	No difference in injury between rapid and slow. Pith and sap wood slightly injured in both.
Rice's Seedling peach twigs	Mar. 22,'13	7½ hrs.	−16.5	
Jonathan apple twigs	Mar. 22,'13	1 hr.	−11.5	Pith injured in both cases. No other tissues injured.
Jonathan apple twigs	Mar. 22,'13	7½ hrs.	−16.5	
Montmorency cherry twigs	Mar. 22,'13	1 hr.	−11.5	Pith injured in both cases.
Montmorency cherry twigs	Mar. 22,'13	7½ hrs.	−16.5	
Chabot plum twigs	Mar. 22,'13	1 hr.	−11.5	Pith and sap wood injured equally in both cases.
Chabot plum twigs	Mar. 22,'13	7½ hrs.	−16.5	

With these twigs it will be seen that the killing temperature of rapidly frozen twigs was four and a half degrees higher than that of the more slowly frozen twigs, and even then the buds of the rapidly frozen twigs killed the worst.

In rapid freezing it required from one to one and three-fourths hours to reach a temperature of -20° C. In slow freezing it required from seven to ten hours to reach the same temperature. Many young fruits and succulent plants were also frozen slowly and rapidly

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but there was so little apparent difference between the results that the data are not given. The killing temperature lies so near the freezing point that possibly the slowly frozen tissue kills badly because it is exposed to temperatures around the killing point longer. This tender tissue was exposed to the minimum temperature for from twenty to thirty minutes.

It will be seen that the rate of temperature fall with winter twigs and buds exerts the greatest influence on the extent of killing at a given temperature of any feature we have so far discussed. And in the case of very forward, rather tender fruit buds, the rate of temperature fall exerts great influence. Thus on March 24, 1913, when all buds, especially of peaches, plums and cherries, had made much growth, a temperature of -11.5° C. killed as many buds with rapid temperature fall as a temperature of -16.5° C. with a slower temperature fall.

Tests were made to see whether the rapid temperature fall that does the most harm is in the early part of the ice forming state, or in the later part. The following table gives the results with peach buds frozen slowly one-half way down to the killling temperature and rapidly the remainder of the way, and others frozen rapidly one-half the way down and slowly the remainder of the way, and others slowly all the way down:

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TABLE 21. SHOWING THE RELATIVE EFFECT ON THE RESISTANCE TO LOW TEMPERATURE OF RAPID TEMPERATURE FALL TOWARD THE BEGINNING AND TOWARD THE END OF THE FREEZING PERIOD.

Kind of Buds	Manner of Freezing	Date	Number of Buds	Percentage Killed
Elberta peach	Slow to −12; fast −12 to −16	Dec. 20,'11	135	3.7
Elberta peach	Fast to −12; slow −12 to −16	Dec. 20,'11	77	71.4
Elberta peach	Slow to −17.5	Dec. 20,'11	129	6.2
Elberta peach	Fast to −16	Dec. 8,'13	135	98.5
Elberta peach	Slow to −12; fast to −16	Dec. 8,'11	113	3.5
Elberta peach	Fast to −12; slow to −16	Dec. 8,'11	135	29.0
Elberta	Medium to −12; fast to −16	Dec. 13,'11	155	52.3
Montmorency cherry	Fast to −12; slow to −20	Feb. 24,'12	142	75.0
Montmorency cherry	Slow to −12; fast to −20	Feb. 27,'12	136	15.4
Montmorency cherry	Fast to −20	Feb. 27,'12	130	96.0
Montmorency cherry	Slow to −20	Mar. 2,'12	163	3.0
Early Richmond cherry	Slow to −12; fast to −20	Mar. 5,'12	291	14.0
Early Richmond cherry	Fast to −12; slow to −20	Mar. 7,'12	283	83.0
Early Richmond cherry	Slow to −20	Mar. 9,'12	297	5.0
Early Richmond cherry	Fast to −20	Mar. 14,'12	263	98.0
Dyehouse cherry	Slow to −12; fast to −20	Mar. 16,'12	184	56.0
Dyehouse cherry	Fast to −12; slow to −20	Mar. 19,'12	200	99.0
Dyehouse cherry	Fast to −20	Mar. 22,'12	150	95.0

It will be seen that rapid falling in the early part of the freezing period down to -12° C., does more harm than rapid fall in the latter part of the period, from -12° C. to the killing temperature. This rapid freezing probably has considerable to do with the amount of killing at times in nature, though just how much it is difficult to tell. In this investigation it was not possible to cause, the temperature to fall more slowly than the most rapid fall to be observed naturally in the climate of this station. Yet there are probably times when on sunny, cold days the temperature of some tissue may rise to near the freezing point due to the absorption of the heat by the dark color of the bark. In this case when the sun is off the twigs, the tempera-

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ture will fall very rapidly. Since rapid temperature fall near the freezing point seems to be more harmful than rapid temperature fall near the killing temperature, it would seem certain that greater killing should thus result. It does not seem impossible that "sun scald" of apple trees may be explained in this way.

This rapid fall of temperature may also be a feature to be considered in heating an orchard. Thus any one who has worked with orchard heaters knows that if on a still night a few of the heaters go out, the temperature will immediately fall to about that which would prevail without the heaters. In this case it is possible that the tissue may kill worse than if the heaters had not been there, since the blossoms or fruit would freeze very rapidly. We can not be certain of this, however, for results with rapid freezing of blossoms at this station have not been uniform enough to be conclusive.

Maturity and Hardiness. Probably the greatest factor in determining the amount of cold that can be withstood by trees and shrubs that live through the winter is a condition of maturity. Emerson[1] has studied the question of maturity of fruit and other trees in Nebraska and has found that the varieties most hardy in wood are those that mature early. Where growth can be checked early in the season, as by a gross feeding cover crop like millet, the trees will also withstand more cold.

Selby[2] made a study of the injury to fruit trees and ornamentals by the severe freeze early in the winter of 1903-4, and attributes the severe injury to the fact that the trees grew late in the fall on account of a very wet period following a period of dry weather.

Eustace[3] reports a study of the effect of the same winter on fruit trees and describes similar conditions. It seems in this case also the great injury is due to the trees' having grown late in autumn.

Winkler[4] found that the resistance of native trees of Germany is least in May, June, July, and August, and gradually increases during September, October, November and December, and is greatest in January, as measured by laboratory freezings.

In summer the fruit buds, for example in August after they can be easily detected, may be killed by a temperature of -9° to -10° C. or somewhat lower on some years. At this time there is little difference between the hardiness of the buds, the wood, and even the foliage, though the foliage kills slightly the worst; while in winter fruit

[1]Nebraska Agr. Exp. Sta. Bul. 79, 1903, (Bibl. No. 33); Neb. Agr. Exp. Sta. Bul. 92, 1906, (Bibl. No. 34); Neb. Agr. Exp. Sta. Anl. Rpt. No. 19, 1906, pp. 101-10, (Bibl. No. 35).
[2]Ohio Agr. Exp. Sta. Bul. 192, 1908. (Bibl. No. 101).
[3]New York (Geneva) Agr. Exp. Sta. Bul. 269, 1905. (Bibl. No. 38).
[4]Jahrb. f. Wiss. Bot. Vol. 52, 1913, pp. 467-506. (Bibl. No. 121).

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buds have been known to survive temperatures of -30° C. and lower, and the tree will survive, under favorable conditions, considerably lower temperature than that: In fact Macoun[1] cites an instance where a Pyrus baccata—Pyrus Malus hybrid—has withstood for five years a climate whose temperature frequently falls to -50° F., and in 1909 it fell twice to -59° F.

At the beginning of winter, as observed by the authors above, the tree tissue generally—whether it is buds, wood, cambium or cortex—will stand less cold than later in the winter. Observation at this station indicates that at least some tissue increases in hardiness rather rapidly for a short time following leaf fall. The following table gives the temperature and result of freezing peach fruit buds, beginning in summer when they are first plainly to be observed and continuing until January:

TABLE 22. SHOWING THE RELATIVE HARDINESS OF FRUIT BUDS AT VARIOUS SEASONS OF THE YEAR.

Kind of Buds	Date	Temperature	Number of Buds	Percentage Killed
Elberta peach buds	July 15,'13	- 6	23	78.2
Elberta peach buds	July 16,'13	- 5	47	100.0
Elberta peach buds	Sept. 15,'11	- 9	186	64.6
Elberta peach buds	Sept. 27,'10	-15	245	39.0
Elberta peach buds	Nov. 1,'10	-16	382	77.8
Elberta peach buds	Nov. 1,'11	- 9	104	60.5
Elberta peach buds	Nov. 14,'11	-12.5	188	69.1
Elberta peach buds	Nov. 12,'09	-13.5	198	1.5
Elberta peach buds	Nov. 17,'11	-14.5	133	100.0
Elberta peach buds	Nov. 18,'11	-12.3	100	91.0
Elberta peach buds	Nov. 18,'11	-13.3	150	99.3
Elberta peach buds	Dec. 1,'09	-22	385	75.3
Elberta peach buds	Dec. 1,'09	-21	343	84.2
Elberta peach buds	Dec. 6,'11	-14.7	225	40.0
Elberta peach buds	Dec. 6,'11	-16	278	56.5
Elberta peach buds	Dec. 14,'09	-20.5	190	74.7
Elberta peach buds	Dec. 18,'09	-22	608	48.2
Elberta peach buds	Jan. 8,'13	-20	105	51.0
Elberta peach buds	Jan. 13,'10	-20	290	97.0
Elberta peach buds	Feb. 23,'10	-19.5	168	63.1
Oldmixon peach buds	Aug. 23,'10	- 8.5	162	93.2
Oldmixon peach buds	Nov. 26,'09	-18	160	81.2
Oldmixon peach buds	Nov. 26,'09	-19.5	229	15.3
Oldmixon peach buds	Dec. 6,'09	-22	290	93.1
Late Duchess apple buds	July 15,'13	- 6	20	70.0
Jonathan apple buds	July 15,'13	- 5	53	73.6
Jonathan apple buds	Nov. 4,'11	-12.5	68	48.5
Jonathan apple buds	Jan. 18,'12	-20.5	35	58.6

[1]Procs. Soc. for Hort. Science, 1912, p. 65. (Bibl. No. 69).

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The following table gives the result of freezing twigs:

TABLE 23. SHOWING KILLING TEMPERATURE OF TWIG TISSUE AT DIFFERENT SEASONS.

Variety	Date	Temperature	Results
PEACHES			
Elberta	July 6,'13	- 6	13 twigs. Cambium dead in 5; cambium and cortex in 8.
Elberta	July 17,'13	- 5	20 twigs. Cambium and cortex dead in all.
Young Champion	July 29,'12	- 5	Twigs 10" long. All tissue injured in terminal 6"; slight injury to cortex only at base of tree.
Champion	Aug. 5,'12	- 5	Bark and cambium killed in all stems.
Belle of Georgia five years old	Aug. 29,'12	- 5	Bark, cambium and sap wood killed in all twigs.
Elberta	Sept. 24,'12	- 5	Bark, cambium, and outer portion sap wood killed.
Elberta, one year old	Oct. 5,'12	- 5	Cambium and cortex injured except at base of one twig. Only injury to sap wood was in terminal part of one twig.
Seedling peach, one year old	Oct. 8,'12	- 5	Twigs injured in cambium and cortex region.
Elberta	Oct. 14,'11	- 9.5	24 twigs. 100% dead. Cambium only killed.
Elberta	Oct. 19,'11	- 5	38 twigs. No injury.
Elberta	Oct. 21,'11	- 7.5	46 twigs. 73.9% dead. Cambium only killed.
Elberta	Oct. 26,'11	- 9	39 twigs. Killing confined largely to cambium.
Elberta	Nov. 1,'11	- 9	23 twigs. 60.6% killed. Cambium only injured.
Elberta	Nov. 2,'11	- 9	51 twigs. 84.3% killed. Cambium only injured.
Elberta	Nov. 16,'12	-15	Slight injury in cortex. Cambium uninjured.
Elberta	Dec. 6,'12	-15	Cortex injured in all; cambium uninjured; pith killed and sap wood occasionally showed injury.
Seedling, one year old	Dec. 18,'12	-16.5	Injured slightly in cortex and pith; cambium and wood sap uninjured.
Seedling, one year old	Dec. 19,'12	-18	Injury confined to pith and cortex.
Elberta	Dec. 7,'12	-16	Injury confined to shoulder below bud and pith region.
Elberta	Jan. 11,'13	-20	Pith dead, other tissues uninjured.
Rice's Seedling	Jan. 25,'12	-19.3	Cortex injured slightly; cambium entirely killed.
Elberta	Mar. 21,'13	-18	Injury to sap wood and pith.
Rice's Seedling	Mar. 21,'13	-18	Injury to sap wood and pith less than with Elberta.
Elberta	Apr. 5,'13	-12.5	Wood uninjured.

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Variety	Date	Temperature	Results
APPLE TWIGS			
Jonathan twigs.....	May 17,'13	- 4	Cortex and epidermis hardiest in a given part. Near base twigs hardier than near tip.
Jonathan twigs.....	May 24,'13	- 4	Slight injury near terminal part.
Jonathan twigs.....	June 7,'13	- 5	Cambium injured throughout all twigs. In terminal 3 in. injury extended to cortex, sap wood and pith. Terminal leaflets more severely injured than remaining ones.
Ben Davis, seven years old.....	June 26,'13	- 4	Severe injury in cambium, cortex and sap wood.
Young Early Harvest.	June 27,'13	- 4	Very slight injury in cambium near terminal.
Early Harvest.....	June 27,'13	- 5	Cambium, cortex, sap wood and pith injured. More severe near terminal.
Ben Davis, five years old.....	July 2,'13	- 4.5	Cambium and cortex severely injured. Sap wood injured.
Late Duchess.....	July 15,'13	- 6	4 twigs; no injury.
Jonathan twigs.....	July 16,'13	- 5	20 twigs; no injury.
Young apple.....	July 27,'12	- 5	All tissue injured 4" back from terminal. No tissue injured farther back than 7" from terminal. Cortex injured most and cambium next.
Jonathan.....	Aug. 15,'12	- 5	Cortex and cambium injured slightly in all samples. Sap wood and pith not injured. Only slight difference between younger and older parts.
Ben Davis.....	Oct. 9,'12	- 8	No parts killed.
Ben Davis.....	Oct. 13,'12	- 9.5	No parts killed.
Jonathan.....	Oct. 16,'12	-15	Cortex killed; cambium uninjured.
Jonathan.....	Nov. 4,'11	-12.5	23 twigs. No injury.
Jonathan, one year old.....	Nov. 16,'11	-15	Slightly injured in cortex. Cambium uninjured.
Jonathan, one year old.....	Nov. 27,'11	-20	38.5 inches of twigs; 67.5% killed in all tissues.
Ben Davis, one year old.....	Nov. 27,'11	-20	40.5 inches of twigs; 14.8% killed in all tissues.
Jonathan, one year old.....	Nov. 29,'11	-19.4	158 inches of twigs; 34.1% killed in all tissues.
Ben Davis, one year old.....	Nov. 29,'11	-19.4	No injury.
Ben Davis, one year old.....	Nov. 29,'11	-21	80 inches of twigs; 7.5% killed in all tissues.
Gano, two years old.....	Nov. 29,'11	-21	38 inches of twigs; 7.8% killed in all tissues.
Jonathan, one year old.....	Dec. 5,'11	-20	136 inches of twigs; 59.8% killed in all tissues.
Gano, two years old.....	Dec. 11,'11	-20.7	113 inches of twigs; 15% killed in all tissues.

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Variety	Date	Temperature	Results
PLUM TWIGS			
Mature plum tree....	July 30,'12	– 5	Cambium, pith and cortex injured throughout. Sap wood slightly injured throughout.
Marianna.....	Oct. 9,'12	– 8	Very slight injury.
Marianna, one year old.....	Nov. 8,'11	–11	New shoots all killed only in spots at basal ends.
Marianna, one year old.....	Dec. 18,'12	–16.5	Injury in cortex and pith. Sap wood and cambium uninjured.
Marianna, one year old.....	Dec. 19,'12	–18	Injury confined to pith and cortex, slight injury to pith. Other tissues uninjured.
Marianna.....	Jan. 11,'13	–20	Slight injury to pith. Other tissues uninjured.

Eustace[1] often observed the greatest injury to peach trees to be just above the snow line. Following the winter of 1904 Green and Ballou[2] reported that an Ohio fruit grower was able to save his trees by banking the bodies with manure, thus keeping the temperature of the trunks near the ground from going as low as that to which the remainder of the tree was exposed.

To test this point at the Missouri Experiment Station, sections of tissue from young and old trees were taken at different points along the trunk beginning near the ground and continuing upward into the branches. These were collected and frozen at intervals throughout the year beginning in September and ending the following July. The results are shown in the following tables:

[1]New York (Geneva) Agr. Exp. Sta. Bul. 269, 1905. (Bibl. No. 38).
[2]Ohio Agr. Exp. Sta. Bul. 157, 1894. (Bibl. No. 48).

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TABLE 24. SHOWING RELATIVE HARDINESS OF DIFFERENT TISSUE.

Variety	Date	Temperature	Location of Tissue	Results
Ben Davis apple tree sections.....	Sept. 4,'12	−5	Sections taken at intervals from near the ground to the twigs.....	Current season's twigs entirely free from injury. In the older wood injury was slight and confined to cambium and outside part of sap wood. The lowest part of trunk had cambium more severely injured than parts higher up.
Sections from eight year old Improved Janet apple tree...	Sept. 11,'12	−6	Sections taken at intervals from near the ground to the twigs.	Injury most severe in cambium region. Younger stems less browned than older. Sap wood injured in 50% of stems, greatest injury on most rapidly thickening side of stem.
Sections from five year old Improved Janet apple tree....	Sept. 12,'12	−6	Sections taken at intervals from near the ground to the twigs.....	Older wood from near ground decidedly more brown than younger wood of twigs. Cortex showed first injury and greatest. Cambium injury slight. Sap wood injury confined to older wood.
Ben Davis apple, seven years old, 2 in. in dia. at crown.....	June 26,'13	−5	Crown (highest point where roots attach 6 in. below surface).....	All sections from lowest to highest injured in cambium. All
Same, 2 in. in dia.....	June 26,'13	−5	6 in. above crown (surface of soil)	

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Variety	Date	Temperature	Location of Tissue	Results
Same, 1¾ in. in dia. at crown.....	June 26,'13	−5	12 in. above crown.	above two feet also injured in cortex. All sections above four feet injured in cambium, cortex and sap wood. The crown was hardiest and the higher up from the crown showed the more injury.
Same, 1⅝ in. in dia...	June 26,'13	−5	18 in. above crown.	
Same, 1½ in. in dia...	June 26,'13	−5	24 in. above crown.	
Same, 1¼ in. in dia...	June 26,'13	−5	36 in. above crown.	
Same, 1 in. in dia...	June 26,'13	−4	48 in. above crown.	
Same, ⅝ in. in dia...	June 26,'13	−5	60 in. above crown.	
Same, ½ in. in dia...	June 26,'13	−5	72 in. above crown.	
Ben Davis apple, five years old, 3 in. in dia.....	July 2,'13	−4.5	Crown.....	Cambium slightly injured.
Same, 2½ in in..... dia.....	July 2,'13	−4.5	6 in. above crown..	Cambium slightly injured.
Same, 2⅜ in. in dia.....	July 2,'13	−4.5	12 in. above crown.	Cambium injured.
Same, 2 in. in dia.....	July 2,'13	−4.5	18 in. above crown.	Cambium seseverely injured.
Same, 1½ in. in dia.....	July 2,'13	−4.5	24 in. above crown.	Cambium severely injured cortex slightly injured.
Same, 1 in. in dia.	July 2,'13	−4.5	36 in. above crown.	Cambium severely injured cortex slightly injured.
Same, 1 in. in dia.....	July 2,'13	−4.5	48 in. above crown.	Cambium and cortex severely injured.
Same, ¾ in. in dia....	July 2,'13	−4.5	60 in. above crown.	Cambium and cortex severely injured; sap wood slightly injured.
Same, ½ in. in dia....	July 2,'13	−4.5	72 in. above crown.	Cambium and cortex severely injured; sap wood slightly injured.
Same, ¼ in. in dia....	July 2,'13	−4.5	84 in. above crown.	Cambium and cortex severely injured; sap wood slightly injured.
Same, ⅛ in. in dia...	July 2,'13	−4.5	96 in. above crown.	Cambium, cortex and sap wood severely injured.

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Variety	Date	Temperature	Location of Tissue	Results
Early Harvest apple, 10 in. in dia..	Sept. 28,'12	−6	Near ground.....	Buds largely killed, bark and cambium taken near ground severely injured. The very youngest wood showed little or no injury. In the wood near the ground, the browning was uniformly distributed. In wood higher up the brown was irregular and in spots. In the young wood, cambium appeared normal—only cortex injured.
Same, 7 in. in dia.....	Sept. 28,'12	−6	48 in. above ground	
Same, 3 in. in dia.....	Sept. 28,'12	−6	96 in. above ground	
Same, 1 in. in dia.....	Sept. 28,'12	−6	12 ft. above ground	
Same, ¾ to ¼ in. in dia.....	Sept. 28,'12	−6	15 ft. above ground (includes this year's growth)	
Jonathan apple, four years old.....	Oct. 19,'12	−7	At ground.....	No injury
Same, four years old..	Oct. 19,'12	−7	12 in. above ground	No injury.
Same, four years old..	Oct. 19,'12	−7	24 in. above ground	No injury.
Same, four years old..	Oct. 19,'12	−7	36 in. above ground	No injury.
Same, four years old..	Oct. 19,'12	−7	48 in. above ground	No injury.
Same, four years old..	Oct. 19,'12	−7	60 in. above ground	No injury.
Same, four years old..	Oct. 19,'12	−7	72 in. above ground	No injury.
Same, four years old..	Oct. 19,'12	−7	84 in. above ground	No injury.
Same, four years old..	Oct. 19,'12	−7	96 in. above ground	No injury.
Jonathan apple, four years old, 2½ in. in dia.....	Oct. 23,'12	−9	At ground.....	All wood, except ¼ in. in dia. or smaller, injured. The most severely browned section was 2 ft. above ground, at the crotch of a rapidly growing branch. The section at the ground was less injured than the one 1 foot above. Cortex, cambium and sap wood injured.
Same, 2⅛ in. in dia.....	Oct. 23,'12	−9	12 in. above ground	
Same, 1⅝ in. in dia.....	Oct. 23,'12	−9	12 in. above ground	
Same, 1 in. in dia....	Oct. 23,'12	−9	36 in. above ground	
Same, 1⅜ in. in dia.....	Oct. 23,'12	−9	24 in. above ground	
Same, .8 in. in dia....	Oct. 23,'12	−9	48 in. above ground	
Same, .5 in. in dia....	Oct. 23,'12	−9	60 in. above ground	
Same, ¼ in. in dia....	Oct. 23,'12	−9	72 in. above ground	
Same, ¼ in. in dia....	Oct. 23,'12	−9	84 in. above ground	
Same, $\frac{3}{16}$ in. in dia.....	Oct. 23,'12	−9	96 in. above ground (Last 4 new growth)	

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Variety	Date	Temperature	Location of Tissue	Results
Jonathan apple, four years old.....	Nov. 14,'12	-12	At ground.....	Most severely injured. Cambium cortex and sap wood injured.
Jonathan apple, four years old.....	Nov. 14,'12	-12	6 in. above ground.	Cambium injured. Cortex slightly injured.
Jonathan apple, four years old.....	Nov. 14,'12	-12	12 in. above ground	Cambium injured. Cortex slightly injured.
Jonathan apple, four years old.....	Nov. 14,'12	-12	18 in. above ground	Cambium injured. Cortex slightly injured.
Jonathan apple, five years old. (Thickest part of trunk, 3 in. in diameter).....	Jan. 15,'13	-20	At crown.....	All tissue injured severely.
Same.....	Jan. 15,'13	-20	3 in. above crown	All tissue injured.
Same.....	Jan. 15,'13	-20	At level of ground.	Slight injury in bark; none in cambium; sap wood injured.
Same.....	Jan. 15,'13	-20	3 in. above ground	Injury in same tissue as above, but not quite so severe.
Jonathan apple, five years old. (Thickest part of trunk, 3 in. in dia.).....	Jan. 15,'13	-20	6 in. above ground.	Same as above.
Same.....	Jan. 15,'13	-20	12 in. above ground	Same as above.
Same.....	Jan. 15,'13	-20	18 in. above ground	No injury in cortex or cambium; sap and heart wood slightly injured.
Jonathan apple, five yearsold. (Thickest part of trunk, 3 in. in dia.).....	Jan. 20,'13	-15.5	At crown.....	All tissue injured severely.
Same.....	Jan. 20,'13	-15.5	3. in. above crown	All tissue injured slightly less than above.
Same.....	Jan. 20,'13	-15.5	At ground.....	Injury in cortex region.
Jonathan apple, five yearsold. (Thickest part of trunk, 3 in. in diameter).....	Jan. 20,'13	-15.5	3 in. above ground	Slight injury in cortex region.

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Variety	Date	Temperature	Location of Tissue	Results
Same	Jan. 20,'13	-15.5	6 in. above ground.	Very slight injury in cortex region.
Same	Jan. 20,'13	-15.5	12 in. above ground	No injury.
Jonathan apple, five years old	Mar. 25,'13	-12.5	At crown	Severe injury in cortex, cambium and sap wood.
Same	Mar. 25,'13	-20	At crown	Very severe injury in cortex, cambium, and sap wood.
Same	Mar. 25,'13	-12.5	3 in. above crown..	Injury in cortex, cambium and sap wood less severe than at crown
Same	Mar. 25,'13	-20	3 in. above crown..	Less severe injury than at crown.
Same	Mar. 25,'13	-12.5	6 in. above crown..	Very slight injury in cortex and cambium.
Same	Mar. 25,'13	-20	6 in. above crown..	Less severe injury than above in same regions.
Same	Mar. 25,'13	-12.5	3 in. above ground.	No injury.
Same	Mar. 25,'13	-20	3 in. above ground.	Very slight injury in cortex.
Same	Mar. 25,'13	-12.5	6 in. above ground.	No injury.
Same	Mar. 25,'13	-20	6 in. above ground.	No injury.
Same	Mar. 25,'13	-12.5	9 in. above ground.	No injury.
Same	Mar. 25,'13	-20	9 in. above ground.	No injury.
Same	Mar. 25,'13	-12.5	12 in. above ground	No injury.
Same	Mar. 25,'13	-20	12 in. above ground	No injury.
Same	Mar. 25,'13	-12.5	18 in. above ground	No injury.
Same	Mar. 25,'13	-20	18 in. above ground	No injury.

It will be seen that the part of the trunk that most slowly develops hardiness on approach of winter is that near the surface of the ground and near the junction of rapidly growing limbs. All the tissue at the lower part of the tree is more tender in early winter than is the upper portion. Of course this might not be true on other seasons. The autumn of 1912, however, was a normal one, the wood apparently going into winter in a well ripened condition. In June and early July the wood in the upper portion of the tree is most tender. It is also interesting to note that in June and July when the

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tissue is generally most tender, the tissue near the base of the tree is most hardy.

Selby[1] observed that the great tenderness in the early part of the winter is probably due to a greater moisture content. He observed that the cambium in winter during the time when plants are very hardy, is in a much dried out condition and in normal seasons has to some extent reached this condition by the time of the early freezes, but in seasons like that one preceding the winter of 1903-4 it is still in a somewhat succulent condition when the early freeze comes.

It is well known that seeds in a dry condition will withstand very much lower temperature than when they have absorbed moisture. Thus Schaffnit[2] reduced the germination percentage of wheat from 100 to 40 by soaking it in water for eight hours at room temperature. In twigs it is probable that a dry condition is essential to the hardiness of the cambium. Shutt[3] and also Allen[4] seem to find a relation between moisture content and hardiness of apple twigs. However, it does not seem that the increase in hardiness of other tissue than cambium, at least of cortex, during early winter can be explained by a decreasing moisture content.

During the winter of 1912-13, beginning November, twigs of apple, peach, plum and cherry were scraped, the cortex ground, weighed carefully, evaporated to dryness in a water bath (to which later glycerine was added to raise the boiling point in the water jacket and thus raise the temperature), and weighed at intervals of two to three days until a constant weight was reached. Samples were taken again in January and again in May. No samples were taken when the tissue was frozen, since then the percentage of moisture would be smaller. The evaporated moisture could not be replaced from below. The following table gives the results:

[1]Ohio Agr. Exp.Sta. Bul. 192, 1908. (Bibl. No. 101).
[2]Mitt. Kaiser Wilhelm Inst. Landw. zu Bromberg, Vol. 3, No. 2, pp. 93-113, 1910. (Bibl No. 98).
[3]Procs. and Trans. Roy. Soc. Canada, ser. 2, Vol. 9, pp. 149-153. (Bibl. No. 104.)
[4]Master's Thesis, Iowa Agr. Exp. Sta. (Bibl. No. 2).

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TABLE 25. SHOWING THE MOISTURE CONTENT OF THE CORTEX IN NOVEMBER, JANUARY AND MAY.

Material	Date	Weight Fresh Sample. Grams	Weight Dry Sample. Grams	Per-cent-age Water	Average Percent-age Water
Jonathan apple, entire twig.	Nov. 15,'12	36.45	17.15	52.95	
Jonathan apple, entire twig.	Nov. 15,'12	35.82	16.75	53.52	53.24
Elberta peach, entire twig....	Nov. 15,'12	26.10	12.35	52.61	
Elberta peach, entire twig....	Nov. 15,'12	28.84	13.85	51.98	52.33
Jonathan apple, buds, bark and cambium.....	Nov. 27,'12	30.05	13.60	54.70	
Jonathan apple, buds bark and cambium.....	Nov. 27,'12	29.95	13.40	55.60	55.2
Elberta peach, buds, bark and cambium.....	Nov. 27,'12	30.15	13.25	56.00	
Elberta peach, buds, bark and cambium.....	Nov. 27,'12	30.00	13.05	56.50	56.3
Jonathan apple, buds bark and cambium.....	Jan. 11,'13	25.00	12.25	51.00	
Jonathan apple, buds, bark and cambium.....	Jan. 11,'13	25.00	12.10	51.60	51.3
Elberta peach, buds, bark and cambium.....	Jan. 11,'13	25.00	10.65	57.40	
Elberta peach, buds, bark and cambium.....	Jan. 11,'13	25.00	11.10	55.60	56.5
Jonathan apple, entire twig.	May 3,'13	20.00	8.96	55.50	
Jonathan apple, entire twig.	May 3,'13	20.00	8.80	56.80	56.15
Elberta peach, entire twig....	May 3,'13	20.00	9.15	54.25	
Elberta peach, entire twig....	May 3,'13	20.00	9.00	55.00	54.63

It will be seen that there is no constant difference in moisture content of the twig cortex from November to May. The difference in the hardiness of the cortex can not be accounted for by the difference in the moisture content, but must be accounted for in some other way. The suggestion is sometimes made that a greater sap density of the twig and other tissue during winter might account for this greater hardiness. It was not possible with our apparatus, at least, to secure sap from the sap wood. The cortex, however, shows this increase in hardiness to a slightly greater extent than does the sap wood. The following table shows the cortex sap density of apple and peach twigs at various seasons of the year, through a period of three years:

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TABLE 26. SHOWING SAP DENSITY OF APPLE AND PEACH TWIGS THROUGHOUT THE YEAR AS MEASURED BY THE FREEZING POINT DEPRESSION.

Date	Elberta peach	Jonathan apple	Gano apple
	Depression	Depression	Depression
January	1.902	2.054	1.630
February	1.841	2.170	No data
March	1.758	No data	1.616
April	1.765	1.055	.949
May	1.055	0.915	1.085
June	1.263	1.415	1.289
July	1.252	1.500	1.469
August	1.652	1.623	1.570
September	1.748	1.605	1.690
October	1.743	1.892	1.728
November	1.765	1.924	1.665
December	1.694*	2.016	No data

*Only one depression taken in December.

It will be seen that while the sap density of the cortex of winter twigs is much greater than that of early summer twigs, yet it is not appreciably greater than that of twigs in late September and October, when the tissue is still considerably more tender than in December and January. Some may suspect that the low sap density of the early summer twigs may be due to their young and somewhat succulent condition. It may be said, however, that the density of the cortex of these young twigs is generally greater than that of any other tree tissue except the leaves. (Data to be published in another bulletin). It would seem certain then that while a part of the increased hardiness of tree tissue in winter may possibly be accounted for by the greater sap density, not all of it can; certainly not the greater hardiness of December tissue over that of October.

In the case of plants killing at as low temperature as those at which winter twigs kill, it seems possible that the sap solute, if it remains in solution, may tend to keep a small amount of water unfrozen and thus protect the protoplasm to some extent. If this should be true, the eutectic point of the sap solute would play a very important part in determining the amount of killing. Some efforts were made to determine whether or not there may be changes in the sap solute as winter comes on that give it a lower eutectic point. Just at the time of leaf fall or slightly before, twigs had the cortex scraped

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from them and the sap expressed in large enough quantities that it could be evaporated down to one-fourth to one-eighth of its volume and leave enough for freezing point determinations with a Beckmann thermometer. The evaporating was done in a dry oven where the temperature never was above 50° C. The evaporating was done in broad, shallow dishes and was generally accomplished in one day. There was apparently no fermentation. In all cases sap taken in October or early November would be thick and gummy long before it could be concentrated to one-sixth of its original volume. It was noticed that it was very difficult indeed to filter the sap from the twigs in autumn or very early winter, while sap taken in December or January or later, filtered much more easily and could be concentrated to one-sixth to one-eight of its volume. In this case it would stay in solution at temperatures as low as could be secured with salt and ice; that is, temperatures low enough to kill many peach buds, indicating that there is certainly a probability that at least a part of the sap solute remains in solution at a temperature low enough to hold water unfrozen to protect the protoplasm. Of course in the earlier season the solidifying of the liquid may be due to colloidal substances in large quantities, and it is entirely possible that the solute had just as low an eutectic point. It was not possible to determine the eutectic point by keeping temperature records since no apparatus was available other than the Beckmann thermometer which could not be used without changing the setting several times, for such low temperature.

It would seem highly probable that, except in the case of cambium, the additional hardiness acquired by the different tissues of the tree as they pass into winter, is a change in the protoplasm such that it can withstand the great loss of water rather than a change in the percentage of moisture or in sap density. It is also possible that changes in the sap solute that lower its eutectic point may occur and that these may increase the resistance to cold by holding water unfrozen to protect the protoplasm from too complete desiccation at lower temperatures.

Rate of Growth and Hardiness. At the time of most rapid growth of deciduous plants in early summer they are generally most tender. Whether this is because they are furthest from the condition of maturity they acquire in autumn and early winter, or because of the very low sap density at this time, it is not easy to say. In some cases the young tissue is most hardy. Thus Goeppert found young leaves more hardy than older ones. Apelt[1] found the

[1]Cohn. Beitrage z. Biol. d. Pfl. Vol. 9, p. 215. (Bibl. No. 3).

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young outer ends of potato shoots to withstand lower temperatures than would the older basal portion. Rein[1] found young onion leaves more hardy than older ones. Winkler[2] found young one-year-old needles of evergreens more hardy than older ones. Shumacher[3] found young yeast cells more hardy than older ones. Fisher[4] found newly formed colloids to regain their normal characteristics after being exposed to a low temperature that would irreversibly change older colloids. On the other hand Bartetzko[5] found that young cultures of *Aspergillus niger* would not withstand as low temperatures as would older ones. It seemed worth while to make some freezings to determine whether or not plant tissues making rapid growth are generally frozen to death at higher temperatures than are tissues growing more slowly. Leaves of various plants were used, leaves that were certainly full grown—in case of those from fruit trees—and leaves that had apparently ceased growing, in case of plants like lettuce, cabbage, kale, etc., were frozen at the same time with young rapidly growing leaves from near the growing tips of the stem. The following table gives the results:

[1]Zeits. f. Naturw. Vol. 80 (1908) p. 1. (Bibl. No. 92).
[2]Jahrb. f. Wiss. Bot. Vol. 52, 1913, pp. 467-506. (Bibl. No. 121).
[3]Sitzungsber der Math. Phys. Klasse d. Wiener Akad. d. Wiss. Alt. 1, 1874. (Bibl. No. 103).
[4]Beitr. Biol. der Pfl. Vol. 10, pp. 133-234, 1911. (Bibl. No. 40).
[5]Jahrb. f. Wiss. Bot. Vol. 47, pp. 57-98 (1911). (Bibl. No. 8).

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TABLE 27. SHOWING THE RELATIVE HARDINESS OF YOUNG RAPIDLY GROWING LEAVES AND OLD MATURE LEAVES.

Material	Date	Temperature	Results
Jonathan apple twigs thawed slowly.....	May 17,'13	-4	2 small terminal leaflets of each of the 4 twigs were injured.
Jonathan apple twigs thawed rapidly.....	May 17,'13	-4	2 twigs had 4 terminal leaflets injured; 1 twig had 5 end leaves injured; 1 twig had 6 end leaves injured. All mature leaves were uninjured.
Jonathan apple.....	May 24,'13	-4	Slight injury in terminal part. Mature leaves uninjured.
Young Jonathan apple.....	June 7,'13	-5	Terminal leaflets injured. Mature leaves green and turgid: slight injury being confined to leaf veins. In terminals, 2 to 5 in. of the twigs, cortex, cambium, sap wood and pith were severely injured, cortex and pith being hardier. Older portion was much hardier.
Jonathan apple.....	June 12,'13	-4.5	In freezer with peach on same date. Injury not so great as that to peach, but younger terminal leaves showed considerable injury while older leaves at the base of the twigs were uninjured.
Salway peach.....	May 24,'13	-4	Very slight injury in terminal part. Mature leaves uninjured.
Elberta peach twigs....	June 7,'13	-5	All terminal buds killed. Mature leaves, including those below 2 in. of terminal, had mid-ribs and veins killed, with other tissues apparently uninjured. Small leaves adjoining large old leaves uninjured. In terminal 3 in. all tissues were injured. Of the remaining portion only the cambium showed severe injury.
Elberta peach.....	June 12,'13	-4.5	Slowly and rapidly growing twigs were used. Only 2 to 3 terminal leaves of the slowly growing twigs showed injury, while on the rapidly growing twigs 7 to 8 terminal leaves were entirely killed Oldest leaves at base of all twigs were uninjured.
Grape shoots.....	May 24,'13	-4	Youngest 10 leaves of 8 in. shoot dead. Mature ⅓ of 18 in. shoot uninjured.

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Material	Date	Temperature	Number Leaves	Percentage Surface Injured	Percentage Leaves all Killed	Depression
Black Seeded Simpson lettuce young leaves...	June 11,'13	−4	18	33.3	16.6	0.520
Black Seeded Simpson lettuce old leaves.....	June 11,'13	−4	40	79.3	70.0	0.575
Black Seeded Simpson lettuce young leaves...	June 12,'13	−4	12	83.3	50.0	
Black Seeded Simpson lettuce old leaves.....	June 12,'13	−4	17	30.8	11.7	
Tomato leaves, old and turgid.....	June 24,'13	−3	32	72.65	50.0	
Tomato leaves, young and turgid.....	June 24,'13	−3	30	63.30	30.0	
Flat Dutch Cabbage, young leaves.....	June 13,'13	−5	10	100.0	100.0	
Flat Dutch Cabbage, old leaves.....	June 13,'13	−5	4	100.0	100.0	
Flat Dutch Cabbage, young leaves.....	June 14,'13	−3	3	0.0	0.0	
Flat Dutch Cabbage, old leaves.....	June 14,'13	−3	4	87.4	75.0	
Red Rock Cabbage young leaves.....	July 1,'13	−4	5	100.0	100.0	
Red Rock Cabbage, old leaves.....	July 1,'13	−4	5	95.0	80.0	
Cowpeas.....	June 14,'13	−3	6 (plants)	Leaves all dead; stems alive. Old and young leaves equally injured. Young stems dead; old stems alive.		
Young tobacco leaves, 2-12 cm. in length.....	July 12,'13	−2	5	20.0		
Old tobacco leaves, 20-40 cm. in length.....	July 12,'13	−2	5	75.0		
Young tobacco leaves, 2-12 cm. in length.....	July 12,'13	−2.5	5	75.0		
Old tobacco leaves, 20-40 cm. in length.....	July 12,'13	−2.5	5	90.0		

In case of apples and peaches the young leaves are uniformly more easily killed, while in the case of some of the succulent plants there is little or no difference. In case of lettuce the young leaves are certainly the more resistant. The density of the sap of the old peach leaves was such that an average of ten freezing point determinations gave a depression of 1.931 while for the young leaves the depression was 1.663. With the Jonathan apple, the depression for old leaves was 1.849 while for young leaves it was 1.202. With these leaves then it seems that the greater sap density will explain the greater

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hardiness of the older leaves. In fact the following table, giving results where twigs with young leaves were placed in cane sugar and glycerine solutions, indicates that increasing the sap density of the young leaves to that of the old leaves will increase their hardiness to nearly that of the old leaves.

TABLE 28. SHOWING RELATIVE HARDINESS OF YOUNG AND OLD APPLE LEAVES AND OF YOUNG LEAVES THAT HAD ABSORBED GLYCERINE AND CANE SUGAR.

Age of Leaf	Treatment	Date	Temperature	Number of Leaves	Percentage all Killed	Percentage Surface Killed	Depression
Old....	Water 30 hrs....	July 8,'13	−4	20	0.0	55.00	1.480
Young.	Water 30 hrs....	July 8,'13	−4	26	50.0	82.70	1.340
Young.	Cane sugar 3 hrs (10%).....	July 8,'13	−4	18	11.1	34.90	1.818
Young.	10 %Glycerine 3 hrs.....	July 8,'13	−4	26	0.0	5.70	3.400
Old.....	Fresh.....	July 12,'13	−4	20	0.0	17.50	.....
Old....	Water.....	July 12,'13	−4	20	0.0	28.70	1.540
Young.	Fresh.....	July 12,'13	−4	19	26.3	48.70	.....
Young.	Water.....	July 12,'13	−4	21	71.4	91.70	1.230
Young.	Cane Sugar.....	July 12,'13	−4	22	27.2	48.90	1.792
Young.	Glycerine.....	July 12,'13	−4	22	22.7	32.90	3.400
Old....	Fresh.....	July 12,'13	−7	20	35.0	46.20	.....
Old....	Water.....	July 12,'13	−7	20	80.0	85.00	1.540
Young.	Fresh	July 12,'13	−7	22	77.7	94.30	.....
Young.	Water.....	July 12,'13	−7	22	100.0	100.00	1.230
Young.	Cane sugar.....	July 12,'13	−7	19	57.9	89.40	1.792
Young.	Glycerine.....	July 12,'13	−7	27	44.1	69.40	3.400

In case of lettuce, however, the depression for old leaves was 0.575, and for young leaves 0.520, and the young leaves are the most resistant to the low temperatures. It is possible that the waxy or oily covering on the surface of the young lettuce leaves increased their resistance to low temperatures. In our experience leaves and fruits dipped in glycerine or paraffine have been uniformly more resistant than have tissues not so treated.

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EFFECT UPON HARDINESS OF PREVIOUS EXPOSURE TO TEMPERATURE SLIGHTLY ABOVE THE KILLING TEMPERATURE.

Closely related to the questions of the relation of maturity to hardiness, and the relation of the rate of growth to hardiness, is the relation of exposure to low temperature, above that at which the plants may kill, to hardiness. In fact these problems are so intertwined that it is difficult, if not impossible, to separate them. Thus in case of the greater hardiness of roots kept in cold storage as compared with those kept in warmer places, unquestionably maturity plays a large part but it is not impossible that exposure to cold also had its effect. However, by referring to Table 14 it will be seen that there was little difference among the killing of roots kept in cold storage at a temperature of 31° F., those kept frozen up in the soil, and those kept at a higher temperature in our basement storage room. The relation of exposure to cold to hardiness of winter buds and wood may also be confused with the rate of temperature fall. This problem will be discussed for peach buds in a later part of this paper. In case of some succulent plants, however, the temperature at which they grew must exert an influence on their hardiness. Thus when cabbage, kale and lettuce were grown out of doors in late autumn or early winter, their hardiness was increased over those grown in the greenhouse more than can be explained by the increased sap density. At least their hardiness was increased more than the same increase in sap density brought about by any other means would increase it. When these plants were grown out of doors in early spring or late autumn, it required a much lower temperature to kill them than was required in June or July. On the other hand, plants like tomatoes or cowpeas are influenced in hardiness but slightly by the temperature at which they grow.

Goeppert[1] found little increase in hardiness due to continuous exposure to low temperature with tender tropical plants, but there was such adaptation with more resistant plants. Apelt[2] found that potatoes kept at a temperature of 22.5° C. four to seven weeks were killed at -2.14° C. while potatoes kept at 0° C. for the same length of time killed at -3.08° C. Rein[3] found that a rather large list of very tender plants kept at a temperature of 8° C were not apprecia-

[1] *Ueber die Wärmeentwickelung in dem Pflanzen, etc*, book, 1830. (Bibl. No. 44).
[2] *Cohn's Beitrage z. Biol. d. Pfl.* Vol. 9, 1907, p. 215. (Bibl. No. 3).
[3] *Zeits. f. Naturw.* Vol. 80, 1908, p. 1. (Bibl. No. 92).

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bly hardier than when kept at a temperature of 20° C., while more resistant plants were considerably more hardy when kept at a low temperature. Fisher[1] found that it required a lower temperature to change the nature of colloids like starch paste that had been kept at low temperatures than to change the nature of colloids kept at a high temperature.

Relative Hardiness of Different Tissues at Different Seasons of the Year. When trees are in a rapidly growing condition, apparently the most tender part of the wood tissue is the cambium and the young cortex, and sap wood cells. However, in winter after the wood has reached its greatest maturity, this is not the case. In fact when severe cold comes, the first tissue to kill seems to be the pith in the case of young twigs, and there will be browning in the sap wood and part of the cortex. In case of the cortex the browning is generally worse in the outer or older cells. This was observed by Eustace[2] on peach trees following the winter of 1903-04. We have often observed the same in artificial freezings we have made, as well as on peach trees badly injured in winter. Peach trees so injured that the sap wood seemed practically all browned have, under favorable conditions, had the cambium form new layers of sap wood surrounding this wood. This injured wood soon becomes entirely dead and the tree depends on the new sap wood formed for conductive tissue. We have also observed dead areas of bark following the winter of 1911-12 when underneath there was healthy new bark and healthy cambium. In these cases, at least, the cortex was more tender than the cambium.

The fruit buds of the peach in late summer during the growing season are generally about as hardy as the cortex and cambium, or sap wood of the twigs, though perhaps slightly less hardy than the same tissues in older wood; while in winter under normal conditions, at least with peaches, the fruit buds are generally somewhat less hardy than any of the wood tissue, with the possible exception of the pith cells. However, in the case of a cold wave that comes on very gradually, say during a period of two or three weeks with a very cold night at the end, some of the buds may survive a temperature low enough to kill the sap wood badly. Thus following the winter of 1904-5, when the temperature at Columbia went to -25° C. after several weeks of very cold weather, nearly all of the peach trees had a few live buds left while the wood was very badly damaged; and in the case of peaches in New York following the winter of 1903-04,

[1]Beitr. Biol. der Pfl. Vol. 10, pp. 133-234, 1911. (Bibl. No. 40).
[2]New York (Geneva) Agr. Exp. Sta. Bul. 269, 1905. (Bibl. No. 38).

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Eustace observed that the trees may be badly injured and yet enough fruit buds left for a full crop of fruit. This last, however, was the condition when the tissue of the tree had not reached the proper maturity before the cold period came. The fruit buds seem, sometimes at least, to reach about their maximum condition of maturity more quickly than wood tissues, especially that near the base of the tree. Tables 23 and 24 give some information as to the relative hardiness of other tissues, and the following table gives the result of some additional freezings where buds were frozen in comparison with other tissues:

TABLE 29. SHOWING RELATIVE HARDINESS OF DIFFERENT TISSUE, INCLUDING BUDS, AT DIFFERENT SEASONS OF THE YEAR.

Variety	Date	Temperature	Results
Elberta peach twigs...	July 15,'13	−6	23 buds, 78.3% killed. 10 twigs 7 injured in cambium. 3 in cambium and cortex.
Elberta peach twigs...	July 16,'13	−5	50 buds, 100% killed. 20 twigs, all injured in cambium and cortex.
Elberta peach twigs, buds and sections of wood.....	July 28,'13	−5	37 buds, 100% killed. Roots, dead in cambium and cortex. Wood just above ground, severely injured in cortex and cambium. 4 feet from ground, same as above. This years' growth, same as above. Sap wood and pith of youngest portions dead.
Champion peach twigs 10 in. long.....	July 29,'12	−5	95% of leaf surface dead. Cortex injured 6 in. back of terminal. Cambium, pith and sap wood injured to 8 in. back of terminal.
Belle of Georgia peach tree, five years old; sections of trunk and limbs.....	Aug. 29,'12	−5	Buds, 100% killed. Young sap wood and cambium killed in all sections. Least injury to bark was in three year old wood. Bark, cambium and sap wood killed in twigs.
Elberta peach tree, buds and twigs and sections of trunk and limbs.....	Sept. 14,'12	−5	Buds 100% injured. Bark cambium and outer portion of sap wood injured in all cases.
Elberta peach twigs (one year old).....	Oct. 5,'12	−5	Buds 90, 91% dead. Cambium and cortex injured except at base of one twig. Sap wood injured only in terminal part twig.
Elberta peach twigs...	Nov. 1,'11	−9	104 buds; 63% dead. 33 twigs; 60.6% killed in cambium, other tissue not injured.
Elberta peach twigs...	Dec. 7,'12	−16	Injury confined to shoulder below bud and pith region. Buds proper, uninjured.

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Variety	Date	Temperature	Results
Elberta peach twigs...	Mar. 23,'13	-18	100 buds, 88% killed. Injury to wood confined in all cases to sap wood and pith.
Rice's Seedling peach twigs.....	Mar. 23,'13	-18	138 buds, 44.2% killed. Injury to wood confined in all cases to sap wood and pith. Injury less than to Elberta.
Late Duchess apple twigs.....	July 15,'13	-6	20 buds, 70% killed. 4 twigs, no injury.
Jonathan apple twigs.	July 16,'13	-5	29 buds, 86.2% killed. 9 twigs, no injury.
Jonathan apple twigs..	July 16,'13	-5	24 buds, 58.3% killed. 11 twigs, no injury.
Young apple twigs, variety not given...	July 27,'12	-5	12 buds, 100% killed. Cortex browned in places, especially around the buds. Cambium dead in region of annual ring and terminal of year's growth. Pith dead in region of annual ring.
Young apple twigs, variety not given....	July 27,'12	-5	Leaves practically all injured. Bark killed about 7 in. back from terminal. Cambium killed about 7 in. back from terminal. Sap wood killed 5 to 6 in. back from terminal. Pith killed 4 to 5 in. back from terminal.
Jonathan apple twigs and wood from three-year old Jonathan tree.....	July 28,'13	-5	44 buds, 70% dead. Roots, cambium and cortex injured throughout. Wood, sections just above ground and 3 feet above dead in cambium and cortex. This year's growth injured in cambium and cortex in older parts, and all tissue dead at tips.
Jonathan apple stems from ⅛ in. to 1 in. in diameter.....	Aug. 15,'12	-5	Cortex and cambium injured slightly in all samples. Sap wood and pith not injured. No marked difference between the different diameters.
Dyehouse cherry young and old wood	July 28,'13	-5	40 buds, 100% killed.
Cherry twigs.....	Aug. 13,'12	-6	25 out of 36 buds killed. Some buds at base of new twigs and on spurs on old wood alive. Leaves all partially injured; only 25% entirely killed. Youngest wood injured worst. Slight injury in cambium and cortex. No injury to sap wood of two year twigs or older.

By referring to this table and to Tables 22, 23 and 24, it will be seen that the fruit buds are in all seasons apparently less hardy than any other tissue, except the pith and the tissue at the base of the buds, and in early winter the wood at the surface of the soil.

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The most tender tissue of the tree is in the roots. Thus in case of the tree frozen July 2, 1913 (Table 24) when the tissue above ground is most tender, the roots were injured in the cambium at -3.5° C., and severely injured in cambium, cortex and sap wood at -4.5° C. Goeppert[1] found that the roots of hardy plants kill at a temperature of from -10° to -15°. A study of the killing temperatures of roots of various fruit trees has been made at this station. In the summer there is not so large a difference between the killing temperature of roots and other tissue. However, the roots do not seem to develop as great maturity as the tissue above ground. The following table shows the results with freezing the roots of trees in summer and winter seasons:

TABLE 30. SHOWING KILLING TEMPERATURE OF APPLE, PEACH, PLUM AND PEAR ROOTS.

Kinds of Roots	Date	Temperature	Results
Two-year Ben Davis apple roots	June 23,'13	-5	Cambium only injured in larger roots. Cortex also injured in roots ¼ in. in diameter. In 2 out of 4 cases, crown uninjured. In other 2 cases there was slight injury to cambium at crown.
Seedling two-year old apple stock roots	June 24,'13	-5	No injury at all
Two-year Ben Davis apple roots	June 25,'13	-3	No injury in roots above ¼ in. in diameter. Wood just above crown was injured in cambium.
Seedling apple stock roots	Aug. 20,'12	-6	Cortex and cambium region all dead.
Seedling apple stock roots	Aug. 22,'12	-5	Cortex and cambium injured, but less severely than above.
Two-year Ben Davis apple roots	Dec. 7,'12	-6.5	Cortex, cambium and wood injured.
Two-year Ben Davis apple roots	Dec. 7,'12	-4	One small root injured.
Seedling apple stock roots from basement since December 20th	Jan. 13,'13	-8	Injury confined to cortex region.
Seedling apple stock roots from basement since December 20th	Jan. 17,'13	-9	Injury slight and confined to cortex region.
Apple stock buried 5 in. below surface outside since Jan. 8th	Mar. 8,'13	-10	One root browned and the other not injured

[1]*Ueber die* Wärmeentwickelung in dem Pflanzen, etc., book, 1830. (Bibl. No. 44).

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Kinds of Roots	Date	Temperature	Results
Apple stock buried 5 in. below surface outside since Jan. 8th	Mar. 8,'13	-14	All roots browned
Two year Ben Davis apple roots	Mar. 24,'13	-10	No injury in first 2 inches; cambium injury throughout remainder. Cortex showed next greatest injury, and in smaller roots sap wood also injured.
Seedling peach roots	June 25,'13	-3	Entire system injured in cambium and sap wood. Injury slightly less in crown.
Seedling peach roots	June 25,'13	-5	All roots very severely injured in cambium and cortex and portion of sap wood. Crown as severely injured as terminal roots. Stem just above ground injured in cortex and cambium.
Elberta peach roots	Oct. 14,'11	-9.5	5 roots; 100% dead.
Elberta peach roots	Oct. 18,'11	-5.5	13 inches of root length; 100% dead.
Elberta peach roots	Oct. 19,'11	-4.5	30½ inches of root length; 100% injured, smaller roots injured worst.
Seedling peach roots	Dec. 7,'12	-6.5	Cortex, cambium and wood injured.
Seedling peach roots	Dec. 7,'12	-4	Slight injury.
Marianna plum root	June 25,'13	-5	Largest root injured severely in cortex. No apparent difference between crown and remainder of root system.
Marianna plum roots.	June 26,'13	-3	Crown 1 in. in diameter shows slight injury in cambium; 3-10 in. down cortex injured also; sap wood also injured towards tips.
Marianna plum roots.	Dec. 7,'12	-4	Slight injury in cortex.
Marianna plum roots.	Dec. 7,'12	-6.5	Cortex, cambium and sap wood injured.
Kieffer pear roots	Oct. 10,'11	-5.5	14½ inches; 65.5% dead.
Kieffer pear roots	Oct. 14,'11	-9.5	5 roots; 100% dead. Twigs at same temperature, cambium only killed.
Kieffer pear roots	Oct. 19,'11	-4.5	33½ inches; 100% injured. Killing more severe in younger roots some distance from the trunk than in larger ones.
Kieffer pear roots	Mar. 27,'13	-10	Injury grading from none in crown to injury of all tissues where diameter of root was not greater than 3-10 inch.

It will be seen that the killing temperature of the roots varies from about -3° C. in summer when most tender to about -12° C. in late winter with rather rapid freezing. The roots are certainly as hardy in March as in January. Thus they are later in becoming tender in spring than are twigs. They are still very tender in autumn

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when tissue above ground has begun to increase rapidly in hardiness. This may be because the soil is still too cold for growth well up into March, generally, and continues warm late in autumn.

The following table gives the result of freezing young apple roots (stock) kept in cold storage at a temperature of 31° to 32° F., in the earth frozen up where the temperature varied from the freezing point to 39° F., and others kept in greenhouse conditions whereby they started into growth, and others kept in basement storage room at a temperature varying from 4° C. to 15° C. from January 8, 1913 to February 16, 1913, the date of freezing.

TABLE 31. SHOWING RELATIVE RESISTANCE TO LOW TEMPERATURES OF APPLE ROOTS KEPT IN DORMANT CONDITION AS COMPARED WITH THOSE IN A GROWING CONDITION.

Kind of Root	Temperature	Number of Roots	Greenhouse	Frozen soil	Basement Storage Room	Cold Storage
Crown cut diameter $\frac{3}{10}$ in.....	−6	2	No injury..	No injury	No injury	No injury
Second cut diameter $\frac{1}{4}$ in.....	−6	2	No injury..	No injury	No injury	No injury
Third cut diameter $\frac{1}{8}$ in.	−6	2	A.Cortex and cambium brown.			
			B.Cambium brown.....	No injury	No injury	No injury
Fourth cut diameter $\frac{1}{16}$ in.....	−6	2	All tissues injured....	No injury	No injury	No injury
Crown cut diameter $\frac{3}{10}$ in.....	−7.5	2	A.Cortex and cambium brown.			
			B.Cambium brown.....	No injury	No injury	No injury
Second cut diameter $\frac{1}{4}$ in.....	−7.5	2	A.Cortex and cambium brown.			
			B.Cambium brown.	No injury	No injury	No injury

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Kind of Root	Temperature	Number of Roots	Greenhouse	Frozen soil	Basement Storage Room	Cold Storage
Third cut diameter $\frac{1}{8}$ in.....	−7.5	2	All tissues injured.....	No injury	No injury	No injury
Fourth cut diameter $\frac{1}{8}$ in.....	−7.5	2	All tissues injured....	Cambium brown... B. No injury.	Cambium brown. B. No injury.	No injury
Crown cut diameter $\frac{3}{10}$ in.....	−9	2	All tissues injured.....	No injury	No injury	A Cambium injured. B. No injury.
Second cut diameter $\frac{1}{4}$ in.....	−9	2	All injured..	No injury	A Cambium injured. B Cambium and cortex injured.	A Cambium injured. B Cambium and cortex injured.
Third cut diameter $\frac{1}{8}$ in.....	−9	2	All injured..	Cambium injured.	All injured	All injured
Fourth cut diameter $\frac{1}{8}$ in.....	−9	2	All injured.	Cambium and cortex injured.	All injured	All injured

Stored Dec. 20th. The second, third and fourth cuts are sections of the stock of equal length below the crown.

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TABLE 31a. SHOWING RELATIVE RESISTANCE TO LOW TEMPERATURES OF APPLE ROOTS KEPT IN DORMANT CONDITION AS COMPARED WITH THOSE IN A GROWING CONDITION.

Kind of Root	Temperature	Number Roots	Outside	Greenhouse
Crown cut diameter 5/16 to ¼ in.	−4	2	No injury....	No injury....
Lower cut diameter ¼ to 3/16 in.	−4	2	No injury....	No injury....
Crown cut diameter 5/16 to ¼ in.	−6	2	No injury....	No injury....
Lower cut ¼ to 3/16 in. in diameter	−6	2	No injury....	No injury....
Crown cut diameter 5/16 to ¼ in.	−8	2	No injury....	One root very brown; other slightly.
Lower cut diameter ¼ to 3/16 in.	−8	2	No injury....	Both roots brown in cortex and cambium.
Crown cut diameter 5/16 to ¼ in.	−10	2	No injury.....	
Lower cut diameter ¼ to 3/16 in.	−10	2	One root brown; other not injured.	
Crown cut diameter 5/16 to ¼ in.	−14	2	All browned...	
Lower cut diameter ¼ to 3/16 in.	−14	2	All browned...	

Stored January 8, 1913.

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TABLE 31b. SHOWING RELATIVE RESISTANCE TO LOW TEMPERATURES OF APPLE ROOTS KEPT IN DORMANT CONDITION AS COMPARED WITH THOSE IN A GROWING CONDITION.

Where Stored	Largest Diameter	Length	Temperature.	Results.
Outside.....	.3 in.....	9+ in...	-9	No injury in first 6 in. from top. Cambium and cortex slightly injured in remainder.
Outside.....	.3 in.....	9+ in...	-9	Cambium injured slightly in last 7 inches.
Outside.....	.3 in.....	8+ in...	-9	Cambium injured slightly in last 7 in; cortex also injured in last 5 in; sap wood injured only at terminal.
Outside.....	.3 in.....	8+ in...	-9	Cambium injured slightly in last 7 in; cortex injured in last 5 in; sap wood injured at terminal.
a)Cold Storage	.3 in.....	7 in.....	-9	No injury in first 5 in; slight injury in cortex and cambium in remainder.
Cold storage...	.25 in.....	9 in.....	-9	No injury in first 6 inches. Cambium injured in remainder.
Cold Storage..	.35 in.....	8 in.....	-9	Cambium injury throughout.
Cold storage...	.3 in.....	7 in.....	-9	Slight injury in cambium throughout.
b)Greenhouse..	.3 in.....	12 in.....	-9	Slight injury in cambium throughout.
Greenhouse....	.25 in.....	10 in.....	-9	Very slight injury in cambium in first 5 inches. Cambium and cortex injury throughout the remainder.

a) In cold storage since January 12, 1913.
b) In greenhouse since January 12, 1913.

TABLE 31c. SHOWING RELATIVE RESISTANCE TO LOW TEMPERATURES OF APPLE ROOTS KEPT IN DORMANT CONDITION AS COMPARED WITH THOSE IN A GROWING CONDITION.

Where Stored	Temperature	Results
Greenhouse since March 29, 1913	-7.5	Injured in cortex, cambium and sap wood throughout.
Cold storage since April 1, 1913..	-7.5	Cambium injured throughout entire root. Cortex showed injury only in the terminal 3 to 5 inches. No injury in sap wood.
Basement store room since December, 1912.....	-7.5	Slight injury.

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It will be seen that there is little difference between the killing temperature of those in storage and those in a storage room at 10 to 37° F. higher temperature and those kept out in the soil. However, those that were in a growing condition were less hardy but with nothing like the difference that would be observed in the case of twigs kept under similar conditions. The reason the roots kept in the basement store room were more hardy than we should expect, is possibly because of their being kept in a somewhat dry condition.

In the case of young peach roots, those kept in cold storage showed a greater hardiness than those kept in the soil outside. Some growth may have taken place in the roots kept out in the soil. The following table gives results of the freezing of peach roots:

TABLE 32. SHOWING RELATIVE RESISTANCE TO LOW TEMPERATURE OF YEAR-OLD SEEDLING PEACH ROOTS GROWING AND IN A THOROUGHLY DORMANT CONDITION.
DATE OF FREEZING, MARCH 22, 1913.

Where Stored	Location of Root	Diameter	Length	Temperature	Results
Outside since January 12, 1913.....	Top even with surface.....	.65 in.....	14 in....	−9	No injury in first 2 in. below the surface of the soil. Cortex, cambium injured in next 4 in. All tissues injured in remainder.
Outside since January 12, 1913.....	2 inches below low surface....	.35 in.....	12 in....	−9	Cambium and cortex injured throughout. Sap wood slightly injured in last 3 in.
Outside since January 12, 1913.....	4 inches below surface....	.3 in.....	12 in....	−9	Cortex and cambium injured severely throughout. Sap wood and pith in last 8 inches.

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Where Stored	Location of Root	Diameter	Length	Temperature	Results
Outside since January 12, 1913.....	6 inches below surface....	.3 in.....	10 in....	−9	Cambium and cortex injured throughout. Sap wood and pith in last 6 inches.
Cold storage... since January 12, 1913.....	Top even with surface.....	.7 in.....	11 in....	−9	Very slight injury of cambium throughout. Cortex slightly in last 2 inches.
Cold Storage since January 12, 1913..	2 inches below surface.....	.3 in.....	9 in....	−9	Very slight injury of cambium throughout. Cortex injured in last 3 in.
Cold storage... since January 12, 1913.....	4 inches below surface....	.35 in.....	8 in....	−9	Cambium and cortex injured throughout. Pith injury last 3 inches.....
Cold storage... since January 12, 1913.....	6 inches below surface....	.3 in.....	6 in....	−9	Cambium and cortex slightly injured throughout. (Injury in all cases very much less than in those kept outside).
Greenhouse since January 12, 1913.....	Top even with surface.....	.7 in.....	11 in....	−9	Very severe injury throughout in cambium and cortex. Pith and wood less severely injured.
Greenhouse since January 12, 1913.....	3 inches below surface.....	.55 in.....	15 in....	−9	Very severe injury in all tissues
Greenhouse since January 12, 1913.....	3 inches below surface.....	.5 in.....		−9	Very severe injury in all tissues.

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The following table gives the results of freezing Marianna plum roots:

TABLE 33. SHOWING RELATIVE RESISTANCE TO LOW TEMPERATURE OF YEAR-OLD SEEDLING PLUM ROOTS GROWING AND IN A THOROUGHLY DORMANT CONDITION.

DATE OF FREEZING, MARCH 22, 1913.

Where Stored	Location of Root	Diameter	Length	Temperature	Results
Outside.....	Top even with surface.....	.8 in.....	16 in....	−9	Very slight injury of cambium in first 10 in. Cortex and cambium injured in remainder.
Outside.....	3.5 inches below surface....	.3 in.....	12 in....	−9	Cambium and cortex injury throughout.
Outside.....	4 inches below surface.....	.3 in.....	14 in....	−9	Cambium and cortex injury throughout.
Outside.....	4 inches below surface.....	.25 in.....	6 in....	−9	Cambium and cortex injury throughout. Pith injured slightly in last 4 inches.
Cold storage...	Top even with surface.....	.8 in. ..	9 in....	−9	Very slight injury in cambium in first 7 in. Cortex and cambium injury in remainder.
Cold Storage...	7 inches below surface.....	.35 in.....	8 in....	−9	Slight injury of cambium and cortex throughout.
Cold storage...	7 inches below surface.....	.35 in.....	7 in....	−9	Slight injury of cambium and cortex throughout.
Greenhouse....	Top even with surface.....	.7 in.....	7 in....	−9	Very severe injury of cortex and cambium throughout. Pith and sap wood injured.

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Where stored	Location of Root	Diameter	Length	Temperature	Results
Greenhouse....	7 inches below surface.....	.45 in.....	10 in....	-9	Very severe injury of cortex and cambium throughout. Pith and sap wood injury.
Greenhouse....	7 inches below surface.....	.35 in.....	3 in....	-9	Very severe injury of all tissue.

In the root system of trees growing out of doors there is great difference in the relative hardiness. The crown of the tree—that is, the part of the root just beneath the ground—will withstand considerably lower temperatures than parts of the root lower down, and the small ends of the roots kill more easily than the larger parts. In fact as the roots extend away from the crown they become more and more tender and apparently this tenderness is greater on those roots that extend downward into the soil. Goff thinks that in Wisconsin the ends of the roots may be killed during every winter. The following table presents data covering this point. The Angers quince roots were frozen on January 25, 1913; the seedling peach roots on March 22, 1913 and the balance on March 24, 1913. See also Table 36 for the same kind of data on plum and cherry roots.

TABLE 34. SHOWING RELATIVE HARDINESS OF VARIOUS PARTS OF THE ROOT SYSTEM OF FRUIT TREES.

Kind of Root	Location of Root	Largest Diameter	Length	Temperature	Results
Two-year Kieffer pear on Japan stock.....	Top even with surface.....	1 in.....	16 in....	-10	No injury in first inch. Cambium injury throughout remainder. Cortex injury last 13 inches. Sap wood in last 9 in.

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Kind of Root	Location of Root	Largest Diameter	Length	Temperature	Results
Two-year Kieffer pear on Japan stock.....	4 inches below surface.....	.5 in.....	12 in....	−10	Cambium and cortex injury throughout. Sap wood injury in last 8 inches.
Two-year Kieffer pear on Japan stock.....	4 inches below surface.....	.45 in....	10 in....	−10	Cambium, cortex and sap wood injured. throughout.
Two-year Kieffer pear on Japan stock.....	4 inches below surface.....	.3 in....	6 in....	−10	Cortex and cambium injured severely throughout; sap wood in last 5 inches.
Kieffer pear roots.....	Top even with surface.....	1.2 in....	15 in....	−10	No injury in first 8 inches; slight injury in cortex and cambium in remainder.
Kieffer pear roots.....	4.5 in below surface.....	.6 in....	12 in....	−10	No injury in first 2 inches; cambium and cortex injury in remainder; pith injury in last 5 inches.
Kieffer pear roots.....	6 inches below surface.....	.4 in....	12 in....	−10	No injury in first 3 inches; cambium and cortex and sap wood injury in remainder.
Kieffer pear roots.....	.3 inches below surface...	.3 in....	8 in....	−10	Cambium and cortex injury throughout. Sap wood injury in last 4 inches..

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Kind of Root	Location of Root	Largest Diameter	Length	Temperature	Results
Two-year Ben Davis apple roots.....	Top even with surface.....	.8 in....	12 in....	−10	No injury in first 2 in. from top. Cambium injury in remainder. Cortex injury in last 3 inches.
Two-year Ben Davis apple roots.....	6 inches below surface.....	.25 in...	10 in....	−10	Slight injury in cambium throughout. Cortex injury in last 2 inches.
Two-year Ben Davis apple roots.....	6 inches below surface.....	.25 in...	8 in....	−10	Cortex and cambium injury throughout. Pith injury in last 5 inches.
Two-year Ben Davis apple roots.....	6 inches below surface.....	.2 in....	6 in....	−10	All tissues injured throughout.
Two-year Ben Davis apple roots.....	6 inches below surface.....	.2 in....	12 in....	−10	Cortex and cambium injury throughout. Sap wood injury in last 6 inches.
Two-year Ben Davis apple roots.....	Top even with surface.....	.8 in....	14 in....	−10	Cambium injury throughout. Cortex injury in last 6 in; sap wood in last 5 inches.
Two-year Ben Davis apple roots.....	5 inches below surface.....	.25 in...	8 in....	−10	Very slight injury in cortex and cambium in last 2 inches.
Two-year Ben Davis Apple roots.....	9 inches below surface.....	.35 in...	8 in....	−10	Cortex and cambium injury throughout Sap wood injury in last 5 inches.

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Kind of Root	Location of Root	Largest Diameter	Length	Temperature	Results
Two-year Ben Davis apple roots.....	11 inches below surface....	.3 in....	6 in....	−10	Cortex and cambium injury throughout. No injury in sap wood.
One-year French apple seedlings.....		.4 in....	12 in....	−10	Cambium injury in last 11 in; cortex injury in last 6 in; pith injury in last 2 inches.
One-year French apple seedlings.....		.4 in....	11 in....	−10	Cambium injury throughout; cortex injury in last 10 in; sap wood injury in last 4 inches.
One-year French apple seedlings.....		.35 in...	8 in....	−10	Cambium slightly injured throughout; cortex injury in last 3 inches.
One-year Japan pear seedlings.....		.4 in....	11 in....	−10	Cambium injury throughout; cortex injury in last 7 inches sap wood injury in last 3 inches.
One-year Japan pear seedlings.....		.4 in....	10.5 in...	−10	Cambium injury throughout; cortex injury in last 8 inches; sap wood injury in last 2 inches.
One-year Japan pear seedlings.....		.35 in...	9 in....	−10	Cambium injury throughout; cortex injury in last 7 inches; sap wood injury in last 4 inches.
Angers Quince roots.....		.5 in....		−7	Cortex slightly injured.

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Kind of Root	Location of Root	Largest Diameter	Length	Temperature	Results
Angers Quince roots.....		.25 in...		−7	Cortex injured.
Angers Quince roots.....		.18 in...		−7	Cortex and cambium injured.
Angers Quince roots.....		.12 in...		−7	Cortex and cambium injured.
Angers Quince roots.....		.5 in....		−9	Cortex injured.
Angers Quince roots.....		.25 in...		−9	Cortex injured.
Angers Quince root.....		.18 in...		−9	Cortex and cambium injured.
Angers Quince roots.....		.12 in...		−9	Cortex and cambium injured.
Seedling Peach roots.....	Top even with surface.....	.65 in...	14 in....	−9	No injury in first 2 inches from top; cortex and cambium slightly injured in next 4 in; all tissues injured in remainder.
Seedling Peach roots.....	2 inches below surface.....	.35 in...	12 in....	−9	Cambium and cortex injury throughout. Sap wood slightly injured in last 3 inches. Injury very slight nearest crown.
Seedling Peach roots.....	4 inches below surface.....	.3 in....	12 in....	−9	Cambium and cortex injured severely throughout. Sap wood and pith injury in last 8 in.
Seedling Peach roots.....	6 inches below surface.....	.3 in....	10 in....	−9	Cambium and cortex injury throughout. Sap wood and pith injury in last 6 inches.

Four-year old Elberta peach roots subjected on October 19, 1911, to a temperature of −4° C., showed both live and dead tissue intermingled. The younger roots lying some distance from the trunk of the tree showed the tissue to be all dead, killing worse than the larger roots near the trunk.

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These roots were in all cases kept during the time preceding the freezing in such a position that all parts must have been exposed to practically the same temperature, so it is not possible that the diminished hardiness of the parts furthest from the crown could have been caused by their being exposed to a higher temperature during the period preceding the freezing. It probably represents the most rapidly growing tissue, but at times of freezing the tissue had not been growing for at least three months. It also represents tissue that under normal conditions is not so liable to be exposed to low temperatures so in the evolution of the plant it would not be so necessary for it to develop hardiness.

Of great interest, practically, is the hardiness of various stocks of fruit trees. Through the courtesy of Mr. E. S. Welch of Shenandoah, Iowa, this station was able to study various stocks. In the case of apple trees worked on French crabs, a considerable number of trees were furnished that had rooted from the scions, as well as from the stock, thus permitting a comparison between these scion roots and the roots from the stock. The trees were received December 20, 1912 and were heeled-in in the shade. The roots were thus at a temperature near the freezing point from the time they were received until they were frozen. The following table gives the results:

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TABLE 35. SHOWING RELATIVE HARDINESS OF STOCK AND SCION ROOTS.

Date of Freezing	Temperature	Material and Results
		Two-year old Ben Davis apple trees.
Feb. 18,'13	-11	Scion root diameter 1/8 in. uninjured for first 6 inches. Cortex and cambium injury in remainder.
Feb. 18,'13	-11	Similar root from stock dia. 1/8 in., uninjured for first 7 inches. Cortex and cambium injury in remainder.
Feb. 20,'13	-15	A. Scion root, largest dia. 3/8 in., length 14 in. No injury whatever.
Feb. 20,'13	-15	B. Scion root, coming from scion 2 inches below A. Largest dia. 1/8 in; length 12 in. No injury in first 5 inches; last 7 inches were slightly browned.
Feb. 20,'13	-15	C. Scion root, 2 inches lower than B. Largest dia. 1/8 in; length 12 in. No injury in first 6 inches; slight injury in last 6 inches.
Feb. 20,'13	-15	D. Stock root, 1 inch below C. Largest dia. 3/8 in; length 10 in. Severe injury throughout.
Feb. 20,'13	-15	E. Stock root, 2 inches below D. Largest dia. 1/2 in; length 10 in. Very severely injured throughout.
Feb. 20,'13	-15	F. Stock root, 1/4 inch below E. Largest dia. 3/8 in; length 12 inches. Severe injury throughout.
Feb. 20,'13	-15	G. and H. Arise at same point as F. Each 1/4 in. in dia. at largest point; both 12 in. long. Severe injury in both.
Feb. 20,'13	-15	Main root. Largest dia. 3/4 in. No injury to scion part. Injury to cortex in stock part.
Feb. 20,'13	-15	Fibrous roots under 1/8 in. dia. Injury on those from stock and not on those from scion.
Mar. 3,'13	-15	A. Scion root 4 inches below ground. Largest dia. 1/2 in; length 10 in. No injury in first 5 inches. Remainder very slightly browned.
Mar. 3,'13	-15	B. Stock root attached 2 inches below A. Largest dia. 1/4 in; length 14 in. Injury throughout entire length.
Mar. 3,'13	-15	C. Stock root 2 inches below B. Largest dia. 1/2 in; length 10.in . Injury in first 4 inches slight; remainder well browned.
Mar. 3,'13	-15	D. Stock root same size as C. Attached at same portion of main root. Injury somewhat more severe than C.
Mar. 3,'13	-15	Main root. Scion part, dia. 3/4 in., shows no browning. Stock part, dia. 1/2 in. is slightly browned.
		Two-year old Wealthy apple trees
Mar. 3,'13	-15	A. Scion root. Largest dia. 1/2 in; length 12 in. No injury in first 7 inches; remainder slightly injured.
Mar. 3,'13	-15	B. Continuation of main root. Largest dia. 3-8 in; length 8 in. No injury in first 3 inches; last 5 inches slightly injured.
Mar. 3,'13	-15	C. Stock root. Largest dia. 1/2 in; length 12 in. Arises nearly opposite Root A. No injury in first 6 inches; remainder slightly injured. No marked difference between A and B.

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In most cases those roots that came from scions were more hardy than those of the same size coming from the stock, indicating that the French seedling roots are less hardy than roots of a variety like Ben Davis. This station has been unable to compare the hardiness of scion roots from hardy varieties like Fameuse with those from very tender varieties like Jonathan.

A comparison similar to the foregoing was made with different stock of plums and cherries using the Myrobolan and Marianna varieties of plums and Mazzard and Mahaleb varieties of cherries. The following table gives the results:

TABLE 36. SHOWING RELATIVE HARDINESS OF MAZZARD AND MAHALEB CHERRY STOCK AND MARIANNA AND MYROBOLAN PLUM STOCK.

Date of Freezing, January 25, 1913.

Kind of Root	Location of Root	Largest Diameter	Length	Temperature	Results
Mazzard cherry roots..		½ in.....		−7	No injury.
Mazzard cherry roots..		¼ in.....		−7	No injury.
Mazzard cherry roots..		3/16 in.....		−7	No injury.
Mazzard cherry roots..		⅛ in.....		−7	Slight injury in cortex.
Mazzard cherry roots..		½ in.....		−9	No injury.
Mazzard cherry roots..		¼ in.....		−9	No injury.
Mazzard cherry roots..		3/16 in.....		−9	Slight browning in cortex.
Mazzard cherry roots..		⅛ in.....		−9	Cortex and cambium injured.
Mahaleb cherry roots..		½ in.....		−7	No injury.
Mahaleb cherry roots..		¼ in.....		−7	No injury.
Mahaleb cherry roots..		3/16 in.....		−7	Cortex injured.
Mahaleb cherry roots..		⅛ in.....		−7	Cortex injured.
Mahaleb cherry roots..		½ in.....		−9	Cortex injured.
Mahaleb cherry roots..		¼ in.....		−9	Cortex and cambium injured.
Mahaleb cherry roots..		3/16 in.....		−9	Cortex and cambium injured.
Mahaleb cherry roots..		⅛ in.....		−9	Cortex and cambium injured.

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Date of Freezing, March 18, 1913.

Kind of Root	Location of Root	Largest Diameter	Length	Temperature	Results
Mazzard cherry roots..	3 inches below surface.....	1.1 in....	7 in....	−10	Cambium and cortex injured throughout. Least severe near crown.
Mazzard cherry roots..	3.5 inches below surface...	.3 in....	5 in....	−10	Cambium and cortex injured throughout. Least severe near crown.
Mazzard cherry roots..	4 inches below surface.....	.3 in....	7 in....	−10	Cambium and cortex injured throughout. More severe than above.
Mazzard cherry roots..	4.5 inches below surface....	.3 in....	12 in....	−10	Cambium and cortex injured severely throughout
Mazzard cherry roots..	5 inches below surface.....	.45 in...	6 in....	−10	Very severe injury in cambium and cortex throughout.
Mazzard cherry roots..	5.5 inches below surface....	.4 in....	10 in....	−10	Very severe injury in cambium and cortex throughout.
Mazzard cherry roots..	5¾ inches below surface....	.28 in...	28 in....	−10	Cortex and cambium injured severely. Pith injured slightly.
Mazzard cherry roots..	6¼ in. below surface.....	.15 in...	8 in....	−10	All regions injured.
Mazzard cherry roots..	6¾ inches below surface....	2 in.....	6 in....	−10	All regions injured.
Mazzard cherry roots..	9¼ in. below surface.....	1 in.....		−10	All regions injured.

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Kind of Root	Location of Root	Largest Diameter	Length	Temperature	Results
Mazzard cherry roots..	2 inches below surface.....	1 in.....	8 in....	−10	Cortex and cambium injury throughout. Least injury near crown. Pith killed last 5 inches.
Mazzard cherry roots..	3 inches below surface.....	.3 in....	6 in....	−10	All tissues severely injured.
Mazzard cherry roots..	4 inches below surface.....	.3 in....	10 in....	−10	Cambium and cortex injured severely first 3 inches of root. All tissues injured in remainder.
Mazzard cherry roots..	4 inches below surface.....	.5 in....	10 in....	−10	Cambium and cortex injury throughout. Pith injury last 5 inches.
Mazzard cherry roots..	4½ inches below surface....	.45 in...	14 in....	−10	Cambium and cortex injured very severely throughout. Pith injured in last 12 inches.
Mazzard cherry roots..	6½ inches below surface....	.4 in....	8 in....	−10	Cambium and cortex injured very severely throughout. Pith injured in last 3 inches.
Mazzard cherry roots..	7½ inches below surface..	.2 in....	6 in....	−10	All tissues injured throughout.
Mazzard cherry roots..	9 inches below surface.....	.15 in...	6 in....	−10	All tissues injured throughout.
Mazzard cherry roots..	10½ inches below surface....	.2 in....	4-6 in...	−10	All tissues very severely injured.
Mazzard cherry roots..	6 inches below surface.....	.15 in...	3 in....	−10	All tissues very severely injured.

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Kind of Root	Location of Root	Largest Diameter	Length	Temperature	Results
Mahaleb cherry roots..	2 inches below surface.....	1 in.....	13 in....	−10	No injury in first 6 in. Very slight injury in cambium in next 3 in. Cambium and cortex injury in remainder.
Mahaleb cherry roots..	8 inches below surface.....	.2 in....	4 in....	−10	Cambium and cortex injury throughout.
Mahaleb cherry roots..	10 inches below surface....	.35 in...	4 in....	−10	Cambium and cortex very slightly injured.
Mahaleb cherry roots..	6 inches below surface.....	.1 in....	4 in....	−10	Cambium and cortex injured severely.
Mahaleb cherry roots..	2 inches below surface....	1 in.....	14 in....	−10	No injury in first 4 inches. Cambium and cortex injured very slightly throughout remainder.
Mahaleb cherry roots..	6 inches below surface.....	.4 in....	6 in....	−10	Cambium and cortex injury throughout.
Mahaleb cherry roots..	10 inches below surface....	.35 in...	2 in....	−10	Slight injury in cambium and cortex.
Mahaleb cherry roots..	9½ inches below surface....	.2 in....	3 in....	−10	Cambium and cortex injured.
	Date of Freezing, March 21, 1913.				
Mazzard cherry roots..	At surface....	1.1 in....	11 in....	−10	Cambium injured throughout. Cortex injury in last 8 in. More severe near terminal; least injury near crown.

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Kind of Root	Location of Root	Largest Diameter	Length	Temperature	Results
Mazzard cherry roots..	3 inches below surface.....	.45 in...	8 in....	−10	Cambium injured throughout. Cortex injured in last 5 in. Least injury nearest crown.
Mazzard cherry roots..	3 inches below surface.....	.3 in....	4 in....	−10	Cambiumslightly injured throughout.
Mazzard cherry roots..	3½ inches below surface....	.4 in....	6 in....	−10	Cambium and cortex injury very slight.
Mazzard cherry roots..	5 inches below surface.....	.3 in....	10 in....	−10	Cambium and cortex injured throughout. Pith injured in last 3 inches.
Mazzard cherry roots..	10 inches below surface....	.3 in....	8 in....	−10	Cambium and cortex severely injured throughout. Pith injured less severely.
Mazzard cherry roots..	At surface....	1.1 in....	13 in....	−10	Cambium injury throughout. Cortex and cambium in last 8 in. Cortex, cambium and pith in last 5 inches.
Mazzard cherry roots..	2 inches below surface.....	.5 in....	10 in....	−10	Cambium injury throughout. Cortex and cambium in last 8 in. Cortex, cambium and pith in last 5 inches.
Mazzard cherry roots..	2 inches below surface.....	.45 in...	8 in....	−10	Cortex and cambium injury throughout.
Mazzard cherry roots..	2½ inches below surface....	.5 in....	5 in.....	−10	Cortex and cambium injury throughout.

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Kind of Root	Location of Root	Largest Diameter	Length	Temperature	Results
Mazzard cherry roots..	3 inches below surface.....	.3 in....	5 in....	−10	Cortex and cambium severely injured throughout. Pith injury in last 4 in.
Mazzard cherry roots..	7 inches below surface.....	.25 in...	5 in....	−10	Cortex and cambium injury throughout. Pith injury in last 3 inches.
	Date of Freezing, March 21, 1913.				
Mahaleb cherry roots..	1 inch below surface....	1 in.....	10 in....	−10	No injury in 5 in. nearest crown. Cortex injury slight in remainder.
Mahaleb cherry roots..	2 inches below surface.....	.15 in...	6 in....	−10	Cambium slightly injured in first 2 in. Cambium and cortex in remainder.
Mahaleb cherry roots..	3 inches below surface.....	.4 in....	8 in....	−10	Cambium and cortex injury throughout. Very slight nearest crown. Pith injury in last 2 inches.
Mahaleb cherry roots..	4 inches below surface.....	.4 in....	6 in....	−10	Cambium and cortex injury slight in first 2 in.; more severe in next 2 in. Cambium, cortex and pith injury in terminal 2 inches.
Mahaleb cherry roots..	5 inches below surface.....	.3 in....	4 in....	−10	Cambium and cortex slightly injured.
Mahaleb cherry roots..	1 inch below surface.....	1 in.....	14 in....	−10	No injury in highest 7 in. Very slight injury in cambium and cortex of remainder.

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Kind of Root	Location of Root	Largest Diameter	Length	Temperature	Results
Mahaleb cherry roots..	4 inches below surface.....	.3 in....	8 in....	−10	No injury in first 4 in.; slight injury in cambium and cortex in remainder.
Mahaleb cherry roots..	4 inches below surface.....	.45 in...	10 in....	−10	No injury in first 3 inches; cambium and cortex injury in remainder; injury becoming more severe near terminal.
Mahaleb cherry roots..	4 inches below surface.....	.5 in....	5 in....	−10	No injury first inch. Cambium and cortex injured in remainder.
Mahaleb cherry roots..	7 inches below surface.....	.3 in....	3 in....	−10	Slight injury in cambium and cortex.
	Date of Freezing, March 18, 1913.				
Myrobolan plum roots....	2 inches below surface.....	.8 in....	7.5 in	−10	Cortex injured first 5 in.; cortex and cambium injured next 2½ inches.
Myrobolan plum roots...	3 inches below surface.....	.4 in....	9.5 in.	−10	Cortex and cambium injured in all. Pith injured in last 5½ inches.
Myrobolan plum roots...	6 inches below surface.....	.2 in....	6 in....	−10	All regions injured, less severe near point of attachment.
Myrobolan plum roots...	9 inches below surface.....	.3 in....	3 in....	−10	All regions injured, less severely near point of attachment.
Myrobolan plum roots...	12 inches below surface..	.25 in...	3 in....	−10	All regions very severely injured.

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Kind of Root	Location of Root	Largest Diameter	Length	Temperature	Results
Myrobolan plum roots...	14 inches below surface....	1 in.....	6 in....	-10	All regions very severely injured.
Myrobolan plum roots...	2 inches below surface.....	.7 in....	12 in....	-10	Cortex injured slightly in first 3 in.; cortex and cambium injured slightly next 6 in.; pith injured slightly and cortex and cambium severely in last 3 inches.
Myrobolan plum roots...	2¾ inches below surface....	.3 in....	6 in....	-10	Cortex injury in first inch. Cortex and cambium injury in remainder, severe near terminal.
Myrobolan plum roots...	3¾ inches below surface....	.2 in....	8 in...	-10	All tissues injured. Injury most severe near terminal.
Myrobolan plum roots...	4¼ inches below surface....	.3 in....	10 in...	-10	All tissues injured. Injury most severe near terminal.
Myrobolan plum roots...	4¾ inches below surface....	.2 in....	9 in....	-10	Same as above.
Myrobolan plum roots...	11 inches below surface....	.35 in...	3 in....	-10	Same as above.
Myrobolan plum roots...	11 inches below surface	.15 in...	3 in....	-10	Same as above.
	Date of Freezing, March 20, 1913.				
Marianna plum roots...	7 inches below surface.....	1 in.....	2 in....	-10	No injury noticeable.
Marianna plum roots...	9 inches below surface.....	.6 in....	12 in....	-10	Slight injury to cambium in first 6 inches; all tissue injured in remainder.

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Kind of Root	Location of Root	Largest Diameter	Length	Temperature	Results
Marianna plum roots...	9 inches below surface.....	.55 in...	6 in....	−10	Cambium injured first 3 inches. Cambium and cortex in remainder.
Marianna plum roots...	9 inches below surface.....	.5 in....	12 in....	−10	Cambium injured first 3 inches. Cortex and cambium next 2 inches; cortex, cambium and pith in remainder.
Marianna plum roots...	9 inches below surface.....	.5 in....	5 in....	−10	Cambium and cortex injured very slightly throughout.
Marianna plum roots...	9 inches below surface.....	.45 in...	10 in....	−10	All tissues injured.
Marianna plum roots...	12 inches below surface....	.2 in....	6 in....	−10	All tissues injured.
Marianna plum roots...	9 inches below surface.....	.4 in....	10 in....	−10	All tissues injured.
	Date of Freezing, March 24, 1913.				
Marianna plum roots...	1 inch below surface.....	1 in.....	14 in....	−10	No injury to first 5 inches; slight injury to cambium next 3 inches; slight injury to cortex in remainder.
Marianna plum roots...	2 inches below surface.....	.4 in....	8 in....	−10	Slight injury in cortex and and cambium throughout. More severe towards tip.
Marianna plum roots...	2½ inches below surface....	.3 in....	10 in....	−10	Same as above.
Marianna plum roots...	2 inches below surface.....	.2 in	8 in....	−10	Same as above.
Marianna plum roots....	6 inches below surface.....	.35 in...	14 in....	−10	Cambium and cortex injured throughout. More severe last 8 in. Pith last 4 in.

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Kind of Root	Location of Root	Largest Diameter	Length	Temperature	Results
Marianna plum roots...	6½ inches below surface....	.15 in...	6 in....	−10	Cambium and cortex injured throughout.
Marianna plum roots...	8½ inches below surface....	.15 in...	4 in....	−10	Cambium and cortex injured throughout.
Marianna plum roots...	10½ inches below surface....	.45 in...	3 in....	−10	Cambium and cortex slightly injured.
Myrobolan plum roots...	1 inch below surface.....	.8 in....	12 in...	−10	Cortex, cambium and pith injured slightly throughout. More severe further from crown.
Myrobolan plum roots...	3 inches below surface.....	.3 in....	10 in....	−10	Cambium and cortex injured severely throughout; pith injured slightly.
Myrobolan plum roots...	4½ inches below surface....	.3 in....	8 in....	−10	Same as above.
Myrobolan plum roots...	5 inches below surface.....	.25 in...	9 in....	−10	All tissues severely injured.
Myrobolan plum roots...	At surface....	.8 in....	10 in....	−10	Cambium, cortex and pith injured in all. More severely near tip.
Myrobolan plum roots...	3 inches below surface.....	.35 in...	8 in....	−10	Cambium and cortex severely injured throughout. Pith injured in last 5 inches.
Myrobolan plum roots...	5 inches below surface.....	.2 in....	4 in....	−10	Cambium and cortex severely injured throughout. Pith injured slightly.
Myrobolan plum roots...	7 inches below surface.....	.2 in....	4 in....	−10	All tissues severely injured.

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Freezing to Death of Pollen. The killing of the bloom and young fruit of the peach, apple and some other fruits will be discussed later in this paper. However, it is perhaps not out of place to discuss here the killing of pollen. Schaffnit[1] exposed the pollen of a number of species of plants to a temperature of -17° C. for eight hours with no apparent harmful effects, while Sandsten[2] apparently reduced the germination percentage of pear, plum, cherry and peach pollen by exposing for six hours to a temperature of 1.5°. At this station pollen of the Jonathan and Cillago apples was frozen with the result shown in Table 37. The pollen was frozen for eighteen hours and then germinated in a 10% sugar solution.

TABLE 37. SHOWING EFFECT OF LOW TEMPERATURE ON GERMINATING POWER OF POLLEN GRAINS.

Kind of Pollen	Date	Temperature	Time at Minimum	Percentage Germination Unfrozen	Percentage Germination Frozen
Jonathan apple.....	Apr. 24,'13	-3	45 min....	84.0	33.0
Jonathan apple..... pollen dried.....	May 29,'13	-13	60 min....	25.0 (estimate)	20.0 (estimate)
Cillago apple.....	May 10,'13	-8	30 min....	66.66	25.0
Cillago apple.....	May 10,'13	-12.5	30 min....	66.66	00.0

Thus the pollen will still germinate after exposure to as low temperature as -8° C., and dried pollen at as low a temperature as -13, a much lower temperature than the other flower parts will withstand.

Discussion of the killing temperature of other flower tissues will be found in a later part of this paper.

Rest Period of Plants. Closely associated with the question of maturity in the fall is that of maintaining a condition of maturity in late winter. Plants that have started into growth in spring soon reach a condition whereby they are as tender as they were in early fall, and sometimes even more so. Under conditions such as prevail in the southern half of Missouri, where growth may continue late in autumn, and where in January and early February, there are likely to be days warm enough for considerable growth to take place, it has

[1] *Mitt.* Kaiser Wilhelm Inst. Landw. Bromberg. Vol. 3, No. 2, pp. 93-115, 1910. (Bibl. No. 98).

[2] *Wis.* Agr. Exp. Sta. Research Bul. 4, pp 163-5. (Bibl. No. 97).

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been found that with the peach, the winter rest period of the fruit buds is an important factor in influencing the amount of killing from cold. By the rest period is meant the period during which the buds will not respond in growth to favorable temperature conditions without special treatment. This rest period is shorter with some varieties than with others, and with all varieties tried at this station the rest period is prolonged later into the winter if the tree makes a vigorous growth and continues growing rather late in the season. They will not, therefore, respond so readily to warm periods that may come in late December or January.

Winter Protection of Buds. The buds of trees in winter are covered with bud scales. Some hold that the insulation formed by these scales will keep the buds from reaching a temperature as low as that of the surrounding air. Wiegand[1] however, found that when a thermometer bulb was carefully inserted in large buds there was no great difference between the rapidity of the fall of temperature on such a thermometer and one with a naked bulb. He, therefore, holds that the bud scales offer little protection in the way of holding heat in the buds, but that their protection is against evaporation. To test this matter at this station the scales were removed from buds of peaches in winter before freezing in the freezer previously described with the results shown in Table 38.

[1]Bot. Gaz. Vol. 41, pp. 373-424. (Bibl. No. 117).

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TABLE 38. SHOWING EFFECT OF REMOVING SCALES FROM THE BUDS BEFORE FREEZING ON THEIR RESISTANCE TO VERY LOW TEMPERATURE.

Variety	Date of Freezing	Temperature	Number of Buds. Scales Off	Number of Buds. Scales On	Percentage Killed. Scales Off	Percentage Killed. Scales On
Elberta	Nov. 12,'09	−15	149	65	2.6	1.5
Elberta	Nov. 12,'09	−15	96	133	2.0	1.5
Elberta	Feb. 23,'10	−18	117	168	47.8	63.6
Elberta, wilted	Dec. 15,'10	−20.2	79	83	59.	81.
Elberta water-soaked	Dec. 15,'10	−15	39	35	64.	71.
Elberta, normally turgid	Dec. 15,'10	−15	89	74	46.	75.
Elberta, wilted	Dec. 23,'10	−21.5	59	57	45.	100.
Elberta, normally turgid	Dec. 23,'10	−16	56	59	55.	76.
Elberta, wilted	Dec. 24,'10	−20.5	70	79	74.	100.
Elberta, normally turgid	Dec. 24,'10	−20.5	75	75	52.	32.
Elberta, water-soaked	Dec. 27,'10	−19.7	60	77	93.	19.
Elberta, normally turgid	Dec. 27,'10	−19.7	57	48	57.	75.
Elberta, water-soaked	Dec. 28,'10	−20.5	69	72	94.	45.
Elberta, normally turgid	Dec. 28,'10	−20.5	54	56	79.	71.
Oldmixon	Nov. 26,'09	−16	100	87	29.	74.7
Oldmixon	Nov. 26,'09	−16	98	83	18.4	73.5
Oldmixon	Nov. 26,'09	−21	80	137	6.2	13.8
Oldmixon	Nov. 26,'09	−21	79	92	2.5	17.4
Oldmixon	Nov. 26,'09	−14	150	151	14.6	62.2
Oldmixon	Nov. 26,'09	−14	86	96	17.4	47.9
Oldmixon	Nov. 26,'09	−21	144	198	81.2	98.9
Oldmixon	Nov. 26,'09	−21	74	76	64.8	96.
Oldmixon	Dec. 6,'09	−22.5	196	132	15.7	54.5
Oldmixon	Dec. 6,'09	−18.5	50	290	40.	93.1
Oldmixon	Jan. 14,'10	−21	185	223	96.6	99.5
Oldmixon	Jan. 15,'10	−21	218	262	75.6	97.7
Oldmixon	Mar. 23,'10	−10	156	140	57.	57.1
Bell's Oct., slowly	Feb. 7,'10	−20	210	107	33.8	83.1
Bell's Oct.	Feb. 8,'10	−20.5	298	239	46.6	63.6
Bell's Oct.	Feb. 15,'10	−20	267	353	100.	97.4
Bell's Oct., rapidly	Feb. 16,'10	−20.5	200	249	100.	100.
Bell's Oct.	Feb. 17,'10	−20	180	200	100.	98.
Bell's Oct., slowly	Feb. 18,'10	−18	121	195	17.3	40.5
Bell's Oct., rapidly	Feb. 19,'10	−18	76	245	82.9	93.0
Bell's Oct.	Dec. 6,'10	−21	69	100	66.	45.
General Lee, slowly	Feb. 26,'10	−20.3	233	220	8.5	13.8
General Lee	Feb. 26,'10	−21	71	111	11.2	19.8
Total number of buds			4430	5078		
Average percentage killed					51.0	68.5

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It will be seen that not only in this case were the scales no protection against the cold, but in actual fact those buds that had the scales removed had very uniformly a smaller percentage killed than those still protected by the bud scales. This is at least true during the most dormant condition in winter. Toward spring when the buds have swelled considerably, during warm days, there seems to be practically no difference between the percentage killed with the scales removed and those with the scales on. This seems to demonstrate conclusively that, at least in the case of peaches, the bud scales do not serve to protect the bud from cold, but as Wiegand points out, the bud scales serve only to protect the bud from loss of water by evaporation.

RELATION OF LOW TEMPERATURE TO PEACH GROWING.

Peach Wood Killing. As mentioned previously, on some years the peach wood is badly injured. Generally this injury follows very severe cold winters with lower temperatures than are necessary to kill the buds. However, on some years the wood has been reported badly injured even when a fair set of bloom followed. It is difficult to say just what weather conditions favor winter killing of peach wood in all cases. In the case of young trees, one to three years old, Emerson[1] found that in Nebraska they are more likely to survive the average winter if forced to mature their wood early in autumn, by using gross feeding cover crops as previously mentioned, though older trees were not benefited by such a practice. Eustace[2] reports that following the severe winter of 1903-04 in New York, peach trees more than seven years of age were killed worse than peach trees about seven years of age or younger, except trees one year in the orchard which killed the worst. Green[3] and Ballou, following the same winter, observed that old trees were killed worse than young trees and that trees kept in a good condition of vigor by use of stable manure, mulch, etc., were in better condition than trees that had been permitted to become weak in their growth. This may have been partly because of the dry weather in early summer followed by wet weather in late summer and autumn. The old trees would have been checked in growth more by the drought and thrown into more succulent growth by the later rains, while the more vigorous young trees would be better able to secure sufficient water and tend to continue

[1]Neb. Agr. Exp. Sta. Bul. 79, 1903. (Bibl. No. 33).
[2]N. Y. (Geneva) Exp. Sta. Bul. 269, 1905. (Bibl. No. 38).
[3]Ohio Agr. Exp. Sta. Bul. 157, 1894. (Bibl. No. 48).

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their growth through the drought, and be just starting into their normal dormant period when the wet weather began. They would, not therefore, be so easily pushed into new growth as if they had passed through a drying out period. At least observation indicates that the weak tree which ceases growth early in the season is most readily pushed into spring-like growth in autumn.

It is probably also true that the young tree would offer less resistance to the movement of sap upward in spring. It would thus be able to secure sufficient water supply to prevent drying out until a new layer of sapwood is formed. The old tree would have a greater leaf surface to evaporate moisture and would offer more resistance to the movement of sap. It would, therefore, seem possible that the young tree might recover from the freeze better, though its injury had been greater than that of the older tree.

In the peach orchards on the Horticultural grounds and at Brandsville and other points in South Missouri, following the severe winter of 1904-05 and again following the severe winter of 1911-12, the trees in a healthy, vigorous condition withstood the low temperature better than trees in a weak condition of growth. At Brandsville and other extreme southern Missouri points, following the freeze of 1911-12, recovery was much more satisfactory from trees that had been fertilized with nitrate of soda than with trees that had not been so fertilized. This was even true with one year old trees which is contrary to the experience of Emerson,[1] but it should be noted that the soil is more conducive to early maturity and the growing season longer in southern Missouri than in Nebraska.

As to the best means of securing recovery of trees from winter injury, a great deal depends upon the nature of the injury. Thus following the winter of 1899, Whitten[2] reports that trees very severely pruned, leaving the branches only a few feet long, recovered much better than trees not headed so severely. Following the severe winter of 1904-05 trees rather severely headed-in recovered better than trees that had only one-third or one-half of the previous season's wood cut off. However, Eustace[3] found that this severe heading back of the trees in New York, following the winter of 1903-04, was very harmful to old trees, though apparently beneficial to young trees. Moderate pruning gave much better results.

[1]Neb. Agr. Exp. Sta. Bul. 79, 1903; (Bibl. No. 33) and Neb. Agr. Exp. Sta. Anl. Rpt. No. 19, 1906, pp. 101-10 (Bibl. No. 35).
[2]Mo. Agr. Exp. Sta. Bul. 55, 1902. (Bibl. No. 115).
[3]N. Y. (Geneva) Exp. Sta. Bul. 269, 1905. (Bibl. No. 38).

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Waite[1] reports that very severe pruning of trees in Michigan and other parts of the North, following the same winter, did not give as good results as light pruning—removing about one-third to one-half of the one year old wood,—and in Missouri, both in the Experiment Station orchard and in orchards at Brandsville and Hollister of southern Missouri, old trees that were severely cut back following the severe winter of 1911-12 did not recover as well as old trees with even no cutting back. The amount of cutting that seemed to have been the most desirable, following either the winter of 1908-09 or 1904-05, in Missouri, was in a large number of cases fatal in 1911-12. At Brandsville a large block of trees that had reached a height of something like fifteen feet had their branches cut back to stubs four or five feet long, and a large percentage of the trees died, after making some slight growth in the spring, while trees with no pruning or very light pruning recovered much better.

Just what the difference is it is hard to say, except that preceding the winter of 1911-12, growth conditions were much the same as those described by Waite, Selby and Eustace for Michigan, Ohio and New York preceding the winter of 1903-04; that is, dry weather in the early part of the season, followed by very wet weather causing a late succulent growth.

In deciding how much pruning to give a tree following a severe winter, one must consider the kind of injury. If the lower part of the tree is very severely injured, as it will be when the tree is forced into late succulent growth following conditions that seriously check its growth earlier, the pruning should probably be such as to remove not more than one-half of the one-year-old wood, while if the injury is distributed throughout the tree, and is not so severe that the cortex and cambium are entirely killed at any point, it would seem highly probable that more severe cutting back—say into two-year-old wood—would be desirable. Such cutting back is often beneficial to the trees even when they do not need it to help them recover from severe freezes. However, very severe cutting back, such as Whitten describes, is probably not the most desirable following any kind of a winter since much less cutting will give entirely satisfactory results and a better tree with more fruit buds for the following season's crop. Cutting back to induce recovery from winter injury has always been more successful at this station on young trees than on old ones and on trees kept vigorous by severe pruning during previous years than on less vigorous trees. In fact if trees are old and neglected, severe cutting back all in one year should always be avoided.

[1]U. S. Dept. Agr. B. P. I. Bul. 51, pp. 15-19. (Bibl. No. 111).

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Eustace reports that trees pruned in January, shortly after a severe freeze, gave much better results than trees pruned in March, probably because a large amount of drying out had been avoided by reducing the top. At this station better results have been secured by the early pruning. Fertilizing the soil with a nitrogenous fertilizer, as nitrate of soda or ammonium sulphate, has, at Brandsville, Missouri, resulted in much more satisfactory recovery from winter injury. Where the fertilizer was applied both the year before and the year following the freeze, very slight permanent injury was to be found when surrounding unfertilized trees suffered great injury.

Hardiest Varieties in Wood. The effect of the condition of growth on the hardiness of peach wood is so great that it is difficult to reach an accurate conclusion as to which are the hardiest varieties. Observations must be on a large number of trees, and through a large enough number of years to include many different seasonal conditions. Hedrick,[1] basing his opinion on replies secured from New York growers, places Crosby, Hills Chili, Stevens Rareripe, Gold Drop and Elberta as most hardy in wood; and from replies from Michigan growers, Hills Chili, Crosby, Gold Drop, Kalamazoo, and Bernard. Elberta, Smock and Salway, considered hardy in New York, were considered tender in Michigan. Wager, Jaques Rareripe, Carman, Belle of Georgia, and Hale's Early were considered above the average in hardiness. Eustace[2] found in New York, following the winter of 1903-04, that Stevens Rareripe, Elberta, Thurber and Salway showed little or no wood injury, while Chinese Cling was the most seriously injured. R. F. Howard, formerly of the Nebraska Experiment Station, states in a letter that Russell[3] is one of the most hardy in wood.

At the Missouri Experiment Station there is a peach orchard that has been exposed to the severe winters of 1898-99, 1904-05 and 1911-12. These trees have had such different treatment that they necessarily went into winter in conditions not equally favorable to withstanding the low temperature. We are, therefore, not able to place the varieties as to hardiness with any degree of accuracy. However, it can be said with certainty that, although Elberta is very tender in bud, its wood is exceeded in its ability to recover from such winters by very few varieties. At least this is true of any but one- or two-year-old trees. Hills Chili, Salway, Bernard and Gold Drop have also proved hardy. Chinese Cling has been probably most

[1] Procs. Western N. Y. Hort. Soc. 1908, p. 180. (Bibl. No. 55).
[2] N. Y. (Geneva) Exp. Sta. Bul. 269, 1905. (Bibl. No. 38).
[3] Yearbook, U. S. Dept. Agr. 1911, p. 429. (Bibl. No. 109).

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tender in wood, although it is rather hardy in bud. The station orchard contains no old trees of the Greensboro or Belle of Georgia varieties but young trees of these varieties, and of Victor and Russell, were among those that recovered best from the effects of the winter of 1911-12.

It is interesting to note that Elberta, Greensboro, Belle of Georgia, Victor and Carman are among the more hardy varieties in wood, and are seedlings of the Chinese Cling, which is one of the few most tender varieties in wood.

Killing Peach Buds. In the case of fully dormant peach buds there is but little difference in the killing temperature of the different parts of the flower. In practically all cases where there is killing at all, all of the flower parts are killed and, in addition to this, generally some of the vascular tissue extending down into the twigs from the base of the buds. This was brought out by careful sectioning of killed buds by Mr. R. G. Briggs.[1] Just how much cold a fully dormant peach fruit bud will withstand, it seems practically impossible to approximate. According to Eustace,[2] the minimum temperature that prevailed in Western New York in the winter of 1903-04 ranged from -10° F. to -15° F. In most orchards of that section there was a normal crop although the wood was badly injured, indicating that under some conditions the bud will actually withstand a lower temperature than will some of the woody tissues.

In a letter from Professor F. A. Waugh, he says that in Massachusetts practically a full crop of peaches has been harvested from trees that had been through a temperature of -20° F. and a partial crop of Greensboro had been secured following a minimum temperature of about -27° F. In a letter from Mr. Elmer B. Parker of Wilton New Hampshire, he states that after a temperature of -14° F. in January, a sudden drop, the Elberta crop was a practical failure while Carman had 50 to 75 per cent of a full crop, and Belle of Georgia and Champion had practically a full crop; and in 1910-11 a temperature of -12° F. in December failed to kill enough buds to prevent trees from yielding a full crop. In these cases the buds had not been started into growth by warm periods.

In the year of 1901-02 all of the buds were killed at the Missouri Experiment Station orchard by a temperature of -23° F. on December 20th. In 1902-03 practically all buds were killed by a temperature of -15° F. on February 17. In 1903-04 buds were killed on all varieties except General Lee, Chinese Cling, Thurber, Carman, Gold

[1]Thesis, University of Missouri, 1912. (Bibl. No. 13).
[2]New York (Geneva) Exp. Sta. Bul. 269, 1905. (Bibl. No. 38).

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Drop, Triumph and Lewis, by a temperature of-14° F. on January 29. During the winter of 1904-05 nearly all the buds were killed, yet practically all trees had a few left alive and Triumph and Lewis a fair crop, following a temperature of -25° F. on February 13th. The following table gives the percentage of buds killed for a number of varieties during the season of 1905-06 and the seasons following up to 1911-12 when all were killed by a temperature of -20° F. During 1912-13 there was no severely cold weather and practically none of the buds were killed.

TABLE 39. SHOWING THE PERCENTAGE OF BUDS KILLED ON THE YEARS 1906 TO 1911 INCLUSIVE.

February 5, 1906. Temperature –3° F.

Variety	Number Buds Counted	Percentage Buds Killed
Lewis	1347	25.9
Chinese Cling	599	30.7
Smock	559	32.9
Early Michigan	721	32.9
Poole's Favorite	901	38.5
Sneed	641	41.4
Carman	617	41.9
Gold Drop	744	42.8
Snow	895	44.2
General Lee	529	46.5
Hills Chili	647	50.3
Salway	675	50.9
Early Tillotson	541	54.7
Krummel October	539	56.5
Alexander	532	56.9
Ortiz	1930	57.1
Triumph	1111	59.1
Rareripe	566	59.3
Crosby	896	60.8
Family Favorite	1012	61.0
Captain Ede	704	63.4
Crawford's Early	994	64.1
Bokhara	513	67.4
Briggs' Red	470	67.4
Elberta	1680	67.8
Oldmixon Cling	2080	69.0
Thurber	519	69.1
Henrietta	592	73.9
Glove	889	76.7
Oldmixon Free	1040	78.0
Fitzgerald	641	80.0
Kalamazoo	1249	80.7
Heath Cling	726	81.6
Champion	608	82.2
Early Bernard	752	82.5
Shipley Red	542	88.0
Yellow St. John	512	88.8
Crawford's Late	677	90.2
Susquehanna	692	96.9

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Variety	Number Buds Counted	Percentage Buds Killed
February 4, 1907. Temperature +1° F.		
Early Tillotson	535	18.8
Gold Drop	765	31.5
Lewis	162	42.5
Oldmixon Cling	1442	44.4
Triumph	642	44.8
Poole's Favorite	1003	54.1
Elberta	409	59.9
Smock	804	61.7
Oldmixon Free	504	90.8
February 2, 1908. Temperature +2° F.		
Crawford's Early	250	18.0
Elberta	977	35.3
Kalamazoo	1104	43.4
Family Favorite	671	44.9
Oldmixon Free	11242	45.7
Ortiz	1177	50.8
Sneed	1662	57.5
Dewey Cling	1204	80.9
January 12, 1909. Temperature −11° F.		
Thurber	975	55.2
Early Michigan	463	63.0
Heath Cling	455	74.7
Hills Chili	493	75.2
Smock	230	78.6
Salway	1864	79.1
Ortiz	2355	80.8
Sneed	333	81, 8
Crosby	520	85.3
General Lee	426	87.7
Family Favorite	843	89.9
Dewey Cling	484	89.9
Champion	550	91.2
Early Bernard	544	92.2
Bokhara	1792	92.2
Triumph	753	93.3
Oldmixon Cling	2994	96.3
Elberta	6634	97.3
Fitzgerald	249	97.7
Crawford's Early	848	98.6
Crawford's Late	464	99.5
Ringold	1030	99.5
Oldmixon Free	997	100.0
Golden Gate	167	100.0

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Variety	Buds Number Counted	Buds Percentag Killed
December 9 and 29, 1909. Temperature −5° F. and −8° F.		
Chinese Cling	75	2.6
Family Favorite	200	3.0
Triumph	174	7.4
Hiley	150	11.3
Sneed	185	12.9
Briggs' Red	100	13.0
Early Michigan	126	14.2
General Lee	150	14.6
Salway	559	14.9
Oldmixon Free	200	15.5
Alton	250	16.4
Rice's Seedling	300	17.0
Oldmixon Cling	200	21.0
Crosby	148	22.9
Elberta	400	24.0
Ortiz	200	27.0
Fitzgerald	100	31.0
Heath Cling	141	31.2
Champion	150	33.3
Crawford's Late	146	44.5
Bokhara	150	51.3
Early Bernard	100	52.0
Kalamazoo	150	52.6
January 3, 1911. Temperature −8° F.		
Triumph	275	29.8
Salway	309	34.9
Henrietta	237	43.4
Krummel October	261	45.9
Crosby	530	52.6
Carman	203	52.7
Heath Cling	234	58.1
Captain Ede	244	58.1
Early Bernard	253	59.2
Sneed	383	61.3
Kalamazoo	269	74.3
Fitzgerald	253	79.0

It will be seen that on January 12, 1909, practically all the buds were killed except on the most hardy varieties by a temperature of -11° F. In fact fewer peaches were borne at Columbia following the winter of 1908-09 than following the winter of 1904-05 when the temperature fell to -25° F. on February 13. A temperature of -12° F. on February 2, 1905, killed only a very small percentage of the buds of such tender varieties as Yellow St. John. It may be said too that a temperature of -15° F. on January 7, 1912, killed practically every peach bud at Koshkonong, Missouri. By referring

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to the adjoining chart, showing maximum and minimum temperature curves from December 1st to the date of killing each year at Columbia and at Koshkonong, and for the year 1908-09 and 1909-10 at Geneva, New York, it will be seen that there was not more warm weather to start the buds preceding the freeze of January 12, 1909 at Columbia or that of January 7, 1912, at Koshkonong, than preceding the freeze of February 13, 1905 at Columbia.

It would hardily seem possible that the buds in either case could have been started into slight growth preceding the freeze. Buds start very slowly even at high temperature early in January. By referring to the chart it will be seen that the low temperature of January, 12, 1909, came suddenly, following high temperature, while that of February 13, 1905, came following forty-two days of rather low temperature. For sixteen days the maximum temperature did not go above the freezing point.

There seems to be two possible explanations for the greater hardiness of the fruit buds during the seasons like that of 1904-05 in Columbia. It is possible that by long exposure to low temperature the buds develop the ability to withstand lower temperatures. Since freezing to death of plant tissue seems to result from withdrawal of water from the cell, the fact that slow drying out of the tissue (see Table 19) increased the hardiness of the buds would lend weight to this theory. The cells would be greatly desiccated during the period of low temperature which preceded the freeze of February 13, 1905.

The other possible explanation of this greater hardiness of the fruit buds in 1905 is the very slow falling of the temperature (see Table 20). It will be remembered that the greatest harm resulted when the rapid temperature fall occurred for the first fifteen degrees below the freezing point, though rapid temperature fall during the last ten or fifteen degrees before the killing temperature was reached also caused greater killing than where the temperature fell slowly from the freezing point (see Table 21). This station was unable in its laboratory experiments to maintain a continuous low temperature that would approximate temperature conditions that prevailed outside when buds withstood such extremely low temperatures as in February, 1905. However, buds of varieties known to show greater resistance on such winters were not killed by temperatures that killed buds of varieties like Elberta, known to be tender. The freezing tests in the laboratory then were tests of the relative hardiness of the buds, either of different varieties or of the same variety differing in hardiness on account of local conditions. Twigs to freeze were obtained from New York, from South Missouri, and from

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Georgia, through the kindness of Professor U. P. Hedrick of the Geneva, New York, Experiment Station; of the Ozark Fruit Farm Company of Brandsville, Missouri; and of Professor H. P. Stuckey of the Georgia Experiment Station. The following table gives the results of our freezings:

TABLE 40. SHOWING THE RELATIVE HARDINESS OF ELBERTA PEACH FRUIT BUDS FROM NEW YORK, SOUTH MISSOURI, CENTRAL MISSOURI AND GEORGIA.

Buds from:	Date	Temperature	Number Frozen	Percentage Killed
Geneva, New York	Nov. 27,'08	-25	55	100
Columbia, Missouri	Nov. 27,'08	-25	63	100
Geneva, New York	Nov. 27,'08	-17.5	121	33.8
Columbia, Missouri	Nov. 27,'08	-17.5	147	68.7
Geneva, New York	Dec. 12,'08	-20.5	152	59.8
Columbia, Missouri	Dec. 12,'08	-20.5	111	37.8
Geneva, New York	Dec. 24,'08	-20.5	129	96.9
Columbia, Missouri	Dec. 24,'08	-20.5	283	67.5
Geneva, New York	Dec. 26,'08	-21.5	195	59.4
Columbia, Missouri	Dec. 26,'08	-21.5	216	33.6
Geneva, New York	Dec. 28,'08	-21	76	81.5
Columbia, Missouri	Dec. 28,'08	-21	194	57.2
Geneva, New York	Jan. 1,'09	-20.5	73	100
Columbia, Missouri	Jan. 1,'09	-20.5	178	57.8
Brandsville, Missouri	Jan. 1,'09	-20.5	95	40.0
Geneva, New York	Jan. 1,'09	-22	85	100
Columbia, Missouri	Jan. 1,'09	-22	158	83.5
Brandsville, Missouri	Jan. 1,'09	-22	110	44.5
Geneva, New York	Jan. 4,'09	-21.5	32	100.
Columbia, Missouri	Jan. 4,'09	-21.5	76	59.2
Brandsville, Missouri	Jan. 4,'09	-21.5	81	34.5
Geneva, New York	Jan. 4,'09	-22	26	100
Columbia, Missouri	Jan. 4,'09	-22	87	78.1
Brandsville, Missouri	Jan. 4,'09	-22	79	35.4
Geneva, New York	Dec. 17,'09	-21	159	83.0
Columbia, Missouri	Dec. 17,'09	-21	282	98.2
Experiment, Georgia	Dec. 17,'09	-21	124	98.3
Geneva, New York	Jan. 19,'10	-19.5	115	91.3
Columbia, Missouri	Jan. 19,'10	-19.5	107	92.5
Experiment, Georgia	Jan. 19,'10	-19.5	84	98.8
Geneva, New York	Feb. 22,'10	-22	130	83.3
Columbia, Missouri	Feb. 22,'10	-22	219	40.6
Geneva, New York	Feb. 25,'10	-21	15	60.0
Columbia, Missouri	Feb. 25,'10	-21	200	20.0
Experiment, Georgia	Feb. 25,'10	-21	82	97.5
Geneva, New York	Feb. 25,'10	-21	17	76.4
Columbia, Missouri	Feb. 25,'10	-21	127	58.2
Experiment, Georgia	Feb. 25,'10	-21	74	94.5
Fifteen freezings Geneva buds, average				81.8
Fifteen freezings Columbia buds, average				67.2
Four freezings Brandsville buds, average				38.6

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It should be said that there were more immature or partially developed buds that were killed by a rather high temperature on the twigs from Geneva than on the twigs from either Columbia or Brandsville, which probably explains the higher percentage of buds killed on the Geneva twigs.

It will be seen that except when the freezings were late in February when buds in Missouri had been started into growth by warm days, the buds from New York were not more hardy than those of the same variety from Columbia. In the preceding chart will be found curves showing maximum and minimum temperatures for Geneva, New York; for Koshkonong, Missouri, six miles from Brandsville, Missouri; and for Columbia, Missouri.

It will be seen that the buds from Geneva, New York, had been exposed to lower temperature and to more continuously low temperature preceding the freezing. Yet when they are thawed before freezing and the temperature fall is equally rapid, there seems to be no difference between their hardiness and the hardiness of buds from central or southern Missouri. It is possible that the buds from New York were started into some growth in transit shipment having been made by express. However, the buds from Bransdville, Missouri, came in the same way and, on account of poor railway connections into Columbia from the South were about as long in coming. If they were not as long in coming, they were kept in a rather warm basement room until the New York twigs came. While the data are not such that absolute conclusions could be drawn, at the same time they certainly seem to suggest that the buds from New York had not acquired appreciably greater hardiness due to their having been previously exposed to low temperature. It seems possible that the buds are more resistant to a low temperature at the end of a long cold period than are equally dormant buds to a low temperature that comes with a sudden drop, not so much because of a greater resistance of the protoplasm brought about by prolonged exposure to low temperature as because of the very slow temperature fall. If this be true, a freeze following a thaw in winter should result in greater injury than if there were no thaw, even if the temperature during the thaw does not go high enough to cause growth. This seems to be the experience of growers in the North. Letters from men in Connecticut, New Hampshire, and the peach regions of Canada state that with them killing of the buds generally occurs when the cold period follows a thaw. It would hardly seem possible that weather warm enough to start growth would be experienced in those sections. It would seem more probable that the killing results from

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the rapid temperature fall, while the greater resistance of the buds following a prolonged cold period is due to the very slow temperature fall with the resulting slow withdrawal of water from the cells, especially while the temperature is still near the freezing point. (See Table 21.)

The condition of the tree when it enters the winter should not be lost sight of in considerations such as the above on the killing from cold in winter. As mentioned previously, maturity plays an important part in the resistance of winter tissue to low temperatures. It may be said, however, that reference is had to the same trees for 1905 as for 1909. They were older in 1909 and were unquestionably as mature. In fact in 1909 there was no apparent difference between the hardiness of buds on the most vigorously growing trees and those growing most slowly in this orchard, indicating that all the trees went into the winter in good condition. That the condition that favors greatest hardiness of buds is one of greatest maturity is apparently common knowledge among peach growers in northern sections. On each such winter in the Missouri Experiment Station orchard these trees that had ceased growing and become dormant rather early the season before carried the largest number of buds through the extreme cold uninjured. The same phenomena were observed in Southwest Missouri following the winter of 1911-12 when there were no warm days to start the buds preceding the extreme cold that killed them. In this case only those trees that were large and matured early and had a large amount of small growth down in the tree had any large percentage of buds to survive. These same phenomena have been observed in orchards on other years in Missouri. However, the average buds on a later maturing tree do not seem to be appreciably less hardy than the average buds on earlier maturing trees. In nearly all cases those buds that survived were buds located on small spurs where there would be a whorl of three or four leaves with only one or two buds on the spur, and there are few such buds on a heavily pruned late maturing tree. It was shown in Bulletin No. 74 of the Missouri Experiment Station that generally the largest percentage of buds to survive cold are on the base of the twigs of mature size; the next largest on these small spurs mentioned above; and the smallest percentage to survive will be on the ends of the twigs. However, with extreme cold that will kill all of the buds at the base of the main twigs or practically all of them, there will often be a few on the spurs; that is, the buds on these spurs vary more as to hardiness than do the buds at the base of the whips. This would naturally be expected since the condition of growth with

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reference to light, etc., will vary more. While the percentage of buds surviving on the tree when enough are killed that practically all left are on these small spurs, would naturally be very small, it should be remembered that only a very small percentage of buds on a large healthy tree would be sufficient for a crop that would be profitable to handle. Under conditions when the buds are killed in a fully dormant state, or are killed after some starting following a warm period coming late enough in the season so that the rest period does not affect the amount of starting, the best plan for handling the trees is to prune them down to a size that can be conveniently handled in spraying, pruning, picking, etc., and to practice a system of pruning and cultivation such that they will cease growing as early as August in central Missouri.

Varieties With Most Hardy Fruit Buds When Fully Dormant. There is a great difference in the degree of cold that different varieties will withstand even when fully dormant. Hedrick gives a list of the hardiest varieties for New York and for Michigan, basing his conclusions on letters received from a large number of New York and Michigan growers. The New York growers name Crosby, Hills Chili, Triumph, Gold Drop, Stevens' Rareripe, and Kalamazoo as being the most hardy in bud, Crosby and Hills Chili being listed by a much larger number of growers than either of the other varieties. Hills Chili, Gold Drop, Crosby, Kalamazoo, and Bernard are the hardiest varieties, according to the opinion of the Michigan growers. Waugh lists Greensboro as one of the hardiest under Massachusetts conditions. Growers in the northern part of Missouri have also found Greensboro probably the hardiest peach in bud that they have grown. Judging from years when the buds were killed without any starting into growth by previous warm periods, Hills Chili, Lewis, Thurber, Gold Drop, Triumph and Crosby have been among the very hardiest in bud in Missouri. Lewis is a seedling of Hills Chili. Mr. R. F. Howard, formerly of the Nebraska Experiment Station, and others in Nebraska place Russell,[1] another seedling of Hills Chili, as one of the very hardiest of peaches in bud, as well as in wood.

In Missouri, besides Greensboro and Thurber, Carman, Belle of Georgia, General Lee, and Chinese Cling, and some other varieties of the Chinese Cling group, have been more hardy in bud even under fully dormant conditions, than the majority of the well known varieties. The Green Twig group, especially Snow and Rice's Seed-

[1]Yearbook, U. S. Dept. Agr. 1911, p. 429. (Bibl. No. 109).

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ling, have been found hardy under the same conditions. Champion is also above the average in hardiness under fully dormant conditions, so far as observations in Missouri indicate.

Of the more tender varieties when fully dormant Hedrick lists, from the opinion of New York and Michigan growers, Crawford's Early, Crawford's Late, Chairs Choice, Reeves' Favorite, and Elberta.

In Missouri these varieties are among the most tender on such years under Missouri conditions, with other peaches of the same type as Golden Gate running even more tender, and Oldmixon Free and Cling and the Heath Cling group, and Fitzgerald and Early Bernard, being slightly more hardy.

Rest Period of Peach Fruit Buds. During some of the seasons mentioned above, especially that of 1905-06, a large percentage of the buds were killed at a high temperature because they had been previously started into growth by warm weather. By referring to the temperature chart it will be seen that on many years in Columbia, and on a large majority of years at Koshkonong in the extreme southern part of Missouri, the temperature for December, January and February will average as high as for those three months in Columbia in 1905-06.

During the writer's observations there has very seldom been a year when buds in the peach section of southern Missouri have not been started sufficiently by February 1 to be killed by a temperature considerably higher than would be required to kill buds in northern Missouri, or certainly in Michigan, New York or New England on the same date.

VARIETIES WITH THE LONGEST REST PERIODS.

Any one who has had experience with a large number of varieties of peaches in a climate like that of southern Missouri has observed that there is a wide difference between the amount of starting by February 1 on different varieties. Thus it will be seen by referring to Table 39 that a smaller percentage of buds were killed on February 5, 1906, by a temperature of -3° F., following a warm period, on General Lee, Chinese Cling and Sneed of the Chinese Cling group, and the Green Twig varieties such as Snow and Ortiz, and on Lewis and Early Michigan of the Hills Chili group, than were killed on varieties like Crawford's Early, Elberta, Fitzgerald and the Heath Cling varieties.

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Experiments at this station have indicated that the reason for this is because these varieties have a longer rest period. Attempts to force the buds of various varieties into growth in the early part of the dormant season, by keeping the twigs in the greenhouse with their bases in water, were published in Bulletin No. 74 of the Missouri Experiment Station. The following table summarizes those results for some of the different types of peaches, giving also the percentage of the buds killed by a temperature of -3° F. on February 3, 1906, when the killing at so high a temperature was due to the buds having been started into growth by previous warm days.

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TABLE 41. PERCENTAGE OF BUDS THAT COULD BE STARTED INTO GROWTH BY DECEMBER 12, AND DECEMBER 22, 1906, ON VARIETIES OF HILLS CHILI, CHINESE CLING, GREEN TWIG AND OTHER GROUPS.

Variety	Percentage of buds killed in 1905-06	Percentage of buds started by Dec. 12, 1906	Percentage of buds started by Dec. 22, 1906
HILLS CHILI TYPE			
Hills Chili	53.0	5.2	No data
Lewis	25.9	0.0	39.0
Early Michigan	32.9	5.7	42.4
Average	37.2	3.6	40.7
CHINESE CLING TYPE			
Chinese Cling	30.7	0.0	0.0
General Lee	46.5	0.0	0.5
Sneed	41.4	0.0	8.0
Carman	41.9	No data	25.0
Connett	40.0	0.0	0.0
Family Favorite	61.0	0.0	7.0
Elberta	67.8	No data	56.0
Average	47.0	0.0	13.8
Average (excluding Elberta)	43.6	0.0	6.7
GREEN TWIG VARIETIES			
Snow	42.0	0.0	12.0
Ortiz	57.1	0.0	5.4
Average	49.5	0.0	8.7
HEATH CLING TYPE			
Heath Cling	81.6	17.5	86.0
Dewey Cling	74.8	36.7	88.0
Hubert Cling	77.2	27.4	85.0
Ringold	83.0	30.0	88.0
Average	79.1	27.9	86.7
OTHERS OF PERSIAN RACE			
Elberta	67.8	No data	56.0
Crawford's Early	64.1	No data	54.1
Crawford's Late	90.2	No data	59.0
Oldmixon Free	78.0	No data	98.0
Foster	87.0	No data	83.0
Crosby	60.9	22.8	90.0
Susquehanna	96.9	10.0	65.0
Wheatland	95.4	No data	86.0
Golden Gate	89.7	17.7	58.0
Triumph	59.1	0.0	7.5
Average	78.9	12.6	65.7

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It will be seen that especially the Chinese Cling group of peaches and the green twig varieties have a long rest period and can not be readily pushed into growth by warm periods earlier than about January 20th, while such varieties as Crawford's Early, Foster and all of the Heath Cling varieties could be much more readily pushed into growth in the early season. Fitzgerald and Early Bernard are not included in these experiments, but experience with them proves that they have as short a rest period as Crawford's Early, and are equally tender under Missouri conditions. Elberta, a Chinese Cling seedling, the other parent of which is thought to be Crawford's Early, has a short rest period like Crawford's Early, while Carman resembles the Chinese Cling in bud habits. Since the publication of Bulletin No. 74, there has been opportunity to observe the pushing during late February on Carman as compared with Elberta in southern Missouri, and uniformly the Elberta pushes much more rapidly during January and February, and even March, than Carman, and on nearly every year it blooms earlier, though in North Missouri where peaches bloom later and the effect of the rest period will thus be entirely eliminated before blooming time, Carman and Elberta are likely to bloom together.

EFFECT OF VIGOR OF THE TREES ON THE REST PERIOD.

It was found as published in Bulletin No. 74, Missouri Experiment Station that prolonging the growth of peach trees in the fall will prolong the rest period so that the fruit buds are not so liable to be killed by cold periods following warm periods. This prolonging the growth in the fall was generally accomplished by pruning the trees severely, in this case two years before those tests were made. This caused them to grow more vigorously throughout the summer and to continue growth later and to hold their leaves late in autumn. While the more vigorous growth continued through the second summer after the pruning, the additional vigor over the unpruned trees was not so great.

To test the effect of this additional vigor on the rest period, twigs gathered on various dates from November 23 to January 13 were forced from two to three weeks in the greenhouse. Following is a summary of the table published in Missouri Experiment Station Bulletin No. 74, giving the result of these tests made during the season of 1906-07. The vigorous, pruned trees are termed "cut back" trees.

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Average percentage started on trees making large growth (cut back)	20.5
Average percentage started on trees making small growth (not cut back)	31.2
Number of varieties in which trees not cut back started first	20
Number of varieties in which trees cut back started first	3
Number in which both started about equally	4

Quoting from the publication referred to:

"It will be seen that only two-thirds as large a percentage of buds started on cut back trees as on trees not cut back. It would be expected then that when ninety per cent of the buds had started on trees making small growth, only sixty per cent of those on trees making a large growth would be started. This is borne out in the above table. If we take the average of buds started on twigs taken December 22nd, or later; that is, when the resting period is nearly ended, we have:—

> "For trees making large growth (cut back) 28.3 per cent started; for trees making smaller growth (not cut back) 48.6 per cent started.

"Taking only those varieties in which one tree had sixty per cent of the buds started, and therefore may be considered to have finished its resting period, we have as an average:—

> "On trees making large growth (cut back) 44.3 per cent of the buds started;
> "On trees making smaller growth (not cut back) 83.4 per cent of the buds started."

Results during the previous season when the difference in vigor of the trees was greater, were even more conclusive though perhaps not enough buds were used in the experiment. Thus with Elberta buds on twigs gathered December 2, 1905, 66 per cent of those from slowly growing trees clearly showed swelling by December 21, while none from the vigorously growing cut back trees showed any swelling. Even when the buds from vigorous trees were counted as started, they had not generally made as much growth as had those from weak trees. It should be said, too, that the trees listed as weak trees were of average vigor when compared with those of most commercial orchards.

Since the publication of that bulletin this station has made observations on the effects of severe pruning and late growth on the rest period of buds in the extreme southern portion of Missouri where there are many days in January and February warm enough to start

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growth. It has been generally found that the buds on weak trees are more forward than on trees that have grown late in the fall. At Doniphan, Missouri (in one of the extreme southern counties) during the spring of 1908 in an orchard owned by a Mr. Neal, old Elberta trees that had been weakened by late summer pruning and others that had not been pruned at all were in full bloom by March 15, while young Elberta trees that had been severely pruned the spring and late winter before were not yet in bloom, being apparently at least one week more backward in blooming than the weakly growing trees. Other orchards in the same community showed similar conditions. It should be said, too, that this was not an abnormally early bloom for extreme southern Missouri.

During the spring of 1910 a number of Elberta peach trees were pruned back very severely in the orchard of the Ozark Fruit Farm Company at Brandsville, Howell County. The following winter was so abnormal that nearly all Elberta trees were almost in full bloom by February 22, when a temperature of 14° F. was experienced. Of course the bloom and some of the unopened buds on such trees were killed. At that time the fruit buds on these severely pruned trees that had grown late in the fall were not open, and they suffered much less injury than did buds on trees not cut back.

Again on March 16 a temperature 20° F. was experienced. Buds on these cut back trees had not yet reached the stage of development that had been reached by trees not so treated by February 22, the temperature between those dates being too low for much growth to take place. The following table gives the percentage of buds killed on the severely pruned and on the unpruned trees by the freeze of February 22, and by the freeze of March 16.

TABLE 42. SHOWING THE PERCENTAGE OF BUDS KILLED AT BRANDSVILLE, MISSOURI, BY THE FREEZES OF FEBRUARY 22 AND MARCH 16, 1911, ON SEVERELY PRUNED AND UNPRUNED TREES.

Treatment	Total Number of buds	Percentage of Buds Killed by Freeze of Feb. 22, 1911	Percentage of Buds Killed by Freeze of Mar. 16, 1911
Not cut back	104	85.6	98.08
Cut back	183	48.1	81.9

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Thus 18.1 per cent of the buds on these cut back trees were still alive after the two freezes. These trees set practically a full crop while trees around them bore not more than one or two peaches to the tree. On the same year, trees heavily pruned the previous spring at Doniphan, Missouri (another southern county), were more than three weeks later in blooming than trees not so treated, as reported by Mr. J. R. Stevens.

The rest period can also be as readily prolonged by fertilizing with a nitrogenous fertilizer, thus prolonging the growth into the fall. In the Brandsville orchard mentioned above, in 1910 a plot of trees was heavily fertilized with ammonium sulphate. One plot of thirty trees received 100 pounds and another plot of sixty trees received 100 pounds. The following table gives the percentage of buds killed on those trees receiving the heavy applications of fertilizer on February 22 and on March 16, and also the percentage killed on adjoining trees not so treated, and on trees fertilized lightly with sodium nitrate in 1909 and in 1910:

TABLE 43. SHOWING THE PERCENTAGE OF BUDS KILLED AT BRANDSVILLE, MISSOURI, BY THE FREEZES OF FEBRUARY 22 AND MARCH 16, 1911, ON TREES FERTILIZED WITH NITROGEN AND TREES NOT SO FERTILIZED.

Treatment	Total Number of Buds	Percentage of Buds Killed by Freeze of Feb. 22, 1911	Percentage of Buds Killed by Freeze of Mar. 16, 1911
Fertilized with Ammonium Sulphate	312	44.6	77.6
Check, not fertilized	188	91.5	98.4
Fertilized with Sodium Nitrate 1½ lbs. to the tree in 1909 and 1 lb. to the tree in 1910	225	80.4	87.1

On the trees fertilized heavily the spring before with ammonium sulphate a percentage of buds large enough for a full crop was left after both of these freezes, and the trees set and ripened a full crop of fruit, while the adjoining trees had nothing. Trees fertilized with sodium nitrate, but not so heavily, did not show such marked results, and yet there were enough buds left for a fair crop, if all the orchard

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had set as much so the activities of the curculio would not have been concentrated on so small an area.

It may be said also that trees on another plot fertilized with sixty-three pounds of sodium nitrate to thirty-six trees in March, 1909, had a smaller percentage of buds killed during the winter of 1909-10 and set a heavier crop in 1910. From these results it is evident that where there are a large number of warm periods in the early part of the winter before the rest period is entirely ended, anything that prolongs the growth in the fall greatly reduces the percentage of buds killed. In fact from the southern tier of counties in Missouri, south, even where the freezes follow warm days as late in the season as February, it will still be a benefit to have the trees grow late in the fall, as witness later blooming of the more vigorous trees mentioned above.

It should be said with reference to the ending of the rest period, that so far as we can tell, this does not occur at a definite time, but buds that will push very rapidly when taken into a warm greenhouse in March can be pushed in January or early February, but not nearly so rapidly; so that whatever the ending of the rest period is, it is certainly a gradual process. While the differences between the rate of pushing on vigorous and weak trees would be smaller following warm days late in the season, say in February, than as early as December and January, yet with trees in extreme southern Missouri, there are some differences to be observed as late as February or March as mentioned above. This was certainly true in the case of blooming of peaches in February, 1911, when the more vigorous trees did not bloom until more than a month later than weak trees bloomed. However, in years when the bloom is rather late there will be little or no difference between the blooming of vigorously growing and weakly growing trees. Thus in the spring of 1913 the full bloom on Elberta trees that were fertilized with sodium nitrate in the spring of 1912 and those that were not, came at practically the same time, which was approximately April 5. Carman, though, was at least four days later in coming into full bloom than Elberta, and Carman from our experience has a longer rest period than Elberta.

As far north as Columbia, Missouri, where the peach bloom is never earlier than March 20, and is usually in April, weak trees have never bloomed earlier than vigorous trees, and varieties like Carman, Chinese Cling, etc., have never bloomed during our observations later than trees with a shorter rest period. Thus neither by forcing late growth to prolong the rest period nor by choosing varieties, such as those of the Chinese Cling group, with long rest periods,

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can the date of blooming often be appreciably affected as far north as Central Missouri. In southern Missouri, on account of the protection from early autumn frosts rendered by the excellent air drainage and on account of its being further south, the trees grow later in autumn and for the same reasons, blooming is likely to occur from a week to occasionally six or seven weeks earlier than in central Missouri. Thus the season there between the beginning of dormancy and blooming time is often short enough that the influence of the rest period may be observed even at blooming time. Trees of varieties with a long rest period or trees that grow late in autumn will bloom later, as in the case of the pruned and fertilized trees mentioned above.

Relation of Thinning the Fruit to Hardiness. Another phase of orchard practice with which it seems it would be possible to influence the rest period, is thinning the fruit. In tests reported in Bulletin No. 74 of the Missouri Experiment Station where trees had one-half of the fruit thinned and the other half left to bear a full crop, out of five trees so treated in each case during the following winter a larger percentage of buds were killed on the side that was not thinned than on the side that was thinned, and where the thinning was very heavy or where all of the fruit was taken off, the difference was very marked. The following table gives the results:

TABLE 44. SHOWING EFFECT OF THINNING ON HARDINESS, BUDS STARTED INTO GROWTH BY WARM PERIOD BEFORE END OF REST PERIOD.

Variety	Treatment	Number of Buds Counted	Percentage of Buds Killed
Seedling	Fruit thinned	1529	18.5
Seedling	Fruit not thinned	1657	58.9
Elberta Seedling	Fruit thinned	1020	31.6
Elberta Seedling	Fruit not thinned	1146	36.7
Oldmixon Cling	Fruit thinned	1442	44.5
Oldmixon Cling	Fruit not thinned	1149	53.4
Poole's Favorite	Fruit thinned	818	41.7
Poole's Favorite	Fruit not thinned	807	52.8
Poole's Favorite	Fruit thinned	1563	40.9
Poole's Favorite	Fruit not thinned	1200	55.4
Average (thinned)			35.4
Average (not thinned)			51.4

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During the season of 1908 this study was continued and we have the results for the freeze of January 12, 1909, where the killing was not caused by cold periods following warm periods, but by cold periods when the buds were fully dormant. In this case there was no difference in the hardiness due to thinning, as the following table shows:

TABLE 45. SHOWING THE PERCENTAGE OF BUDS KILLED ON THINNED AND UNTHINNED LIMBS OF TREES WHEN THE BUDS HAD NOT BEEN STARTED INTO GROWTH BEFORE THE FREEZE.

Variety	Date	Number of Buds Thinned	Percentage of Buds Killed. Thinned.	Number of Buds Not Thinned.	Percentage Buds Killed. Not Thinned.
Salway	Jan. 12,'09	345	74.5	425	84.7
Ringold	Jan. 12,'09	397	99.8	289	98.6
Family Favorite	Jan. 12,'09	274	90.8	569	89.1
Elberta	Jan. 11,'09	323	99.4	334	94.4
Elberta	Jan. 12,'09	613	85.3	454	86.5
Elberta	Jan. 12,'09	538	99.9	608	100.0
Triumph	Jan. 12,'09	292	92.1	242	90.9
Magnum Bonum	Jan. 12,'13	308	100.0	250	99.2
Crawford's Early	Jan. 12,'09	412	99.2	436	97.9
Alexander	Jan. 12,'09	230	94.7	194	91.2
Oldmixon Free	Jan. 12,'09	152	100.0	182	100.0
Hills Chili	Jan. 12,'09	397	94.9	405	95.0
Bonanza	Jan. 12,'09	421	95.9	352	96.8
Early Michigan	Jan. 12,'09	145	84.8	496	82.8
Early Michigan	Jan. 12,'09	243	78.6	154	79.8
Total number buds		5090	.....	5390	
Average		.....	93.2	.....	92.5

During the same season artificial freezings of buds from the thinned and unthinned sides of these trees were run, and in this also there was no constant difference between the percentage of buds killed from thinned and unthinned limbs. These results suggest that thinning has its effect on the rest period rather than on the intrinsic hardiness of the buds. Where the tree is bent under a heavy load and under the strain of bearing a heavy crop, as when it is not thinned, the moisture supply probably being partially shut off, the same condition will prevail, at least to some extent, as when the trees are not cultivated; they will become dormant earlier and end their rest period earlier. Thus thinning, like heavy pruning and fertilizing with nitrogen can be expected to increase the hardiness of peach

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fruit buds only in climates like that from Central Missouri South, where there is likely to be weather warm enough to start the buds into growth before the effect of the rest period ends.

It may be said also, especially concerning thin soils such as are to be found in the Ozark regions of Missouri and Arkansas, if the tree is permitted to bear an exceptionally heavy crop, it sets a much smaller number of fruit buds. While experiments have not always indicated that this is true with apples, any one who has had an opportunity to observe a considerable number of peach orchards where only part of the trees have been thinned will be readily convinced that it is true with peaches. This fact has its bearing on the problem of killing from cold since with a very light set following a heavy crop, if as many as 75 per cent of the buds are killed, there will not be enough buds for a good crop, while if the trees were thinned, 25 per cent of the buds would be enough for a heavy crop. By consulting Table 39 it will be seen that in a large number of years as many as 75 per cent of the fruit buds are killed on Elberta trees. In sections like South Missouri where the rest period seriously affects the amount of bud killing, thinning, when necessary, would thus seem to affect the size of the following crop on nearly every year.

The greater hardiness in late winter of fruit buds on severely pruned trees and on trees fertilized with nitrate, like those mentioned above, represents a difference in time of ending the rest period between fruit buds on very vigorous trees and on trees of medium vigor. Where the rest period was prolonged by thinning, the difference in hardiness represents a difference in time of ending the rest period between fruit buds on trees of medium vigor and on trees in a rather weak condition. In case of trees making a weak growth for any reason, as compared with trees making a moderately vigorous growth, so far as our observations have gone, the differences while not so great, are yet often apparent. In a number of years the buds or blossoms have been so nearly all killed that the crop in extreme South Missouri on weakly growing trees, amounted to nothing, while trees of medium vigor, like an average six-year-old tree, bore good crops in the same years.

In attempting to increase the hardiness of peach buds under the climatic conditions prevailing in southern Missouri, by largely increasing the vigor of growth over the average growth, several limiting factors must be considered. In the case of pruning, such large increase in vigor of growth can practically be secured only following winters or springs when the fruit buds or blossoms have been killed, since this cutting back so severely in other years would cause

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the loss of the crop following the spring it is done. However, in the Missouri peach section on all except the high land, at least, the crop is likely under average conditions to be lost probably half of the time and in such years it certainly is advisable to give the trees rather severe cutting back. In none of the experiments above mentioned, however, has it proved an advantage to cut the limbs back so severely that only short stubs, say two or three feet long, are left. In this case the growth will be so vigorous and there will be so much shade that very few fruit buds will form, during the following summer. The tree will also be so greatly reduced in size that even though a crop may be secured the year following, there will be bearing surface for only a small crop. Cutting back into two-year-old wood or sometimes into three-year-old wood and shearing the small growth off the limbs so that there will be only stocky, vigorous twigs to form in summer, has secured the best results. The cutting back should be light enough that buds will form practically to the base of all of the new twigs. With pruning like this a large bearing surface is left and a large number of buds generally set. Observation has uniformly indicated that following periods when part of the buds are killed, most of the fruit will be borne on the twigs down along the limbs rather than on the very vigorously growing twigs that form along the top of the limbs. No such twigs will be formed along the limbs where the tree has been given too heavy pruning as where the limbs are left only three to five feet long.

For these southern Missouri conditions the pruning in years when there is a crop should be as severe as can be given and yet secure a maximum crop of the best quality of fruit from the tree. If this is practiced there will be later pushing of buds the following winter and in some years a slightly later blooming on trees so pruned than on trees that have had less pruning. However, where the soil is very thin it may be impossible to keep the trees in a sufficiently rapidly growing condition to secure a prolonged rest period without too greatly reducing the size of the tree. In this case the vigor should be kept up by a combination of pruning, cultivation and fertilizing with nitrogen (or a complete fertilizer if it is proved that other elements than nitrogen are needed in the given soil).

In fertilizing with nitrogen, however, to keep up the vigor of the trees, caution should be used because heavy applications of nitrogen-bearing fertilizers seriously injure the color of the fruit borne the summer following, and cause fruit to rot. This has been our experience each year at Brandsville, Missouri. Increasing the vigor by pruning does not have this effect to any appreciable extent, but generally

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tends to improve the quality of the fruit by increasing its size. Fertilizing with nitrogen should be as light as it is possible to give and yet keep up proper vigor and size of the tree by combining nitrogen fertilizing with pruning and good cultivation. This may be said, however, that since it has become the regular practice to spray peaches, this injury to color is not so serious as it formerly was because the spraying burns into the peach generally a rather brilliant color. If in addition to spraying the fruit is thinned and the trees kept well open, the injury to color and diminished resistance to rot from the use of nitrogen is not so great.

Probably under average conditions a good system to follow would be to fertilize with the equivalent of one or one and one-half pounds of sodium nitrate to the tree and to prune as much as possible without reducing the crop of high-grade fruit in any year. The nitrogen should be applied not every year but only often enough to maintain the desired size of the tree, since the pruning has a dwarfing effect. It may be observed that inducing vigorous growth will cause a late growth in autumn; and that therefore the fruit buds and wood will not be in a desirably mature condition for winter. This is certainly true in some northern sections and in case of buds has in several years since 1901 been true in Columbia. Since 1901 there has probably been only one winter when a larger percentage of buds were killed on late growing than on early maturing trees in extreme southern Missouri, and even in that year-1911-12—the wood of the vigorous trees best recovered from the effects of the winter. It should be remembered that in southern Missouri the soil is so light and the season so long that it is not easy to prolong the growth of a peach tree more than two years from planting enough to prevent its going into winter in the best condition for that section.

It may be said that before recommending a method like this for increasing the hardiness of buds, it probably should be tested out during a period of ten or twelve years so that observations will extend through enough seasons that one may be sure he has struck an average. However, this may be said, that the methods here recommended are certainly the best for the orchard, regardless of the effect on the hardiness of the tree. Fertilizing with nitrogen has caused the tree to resist certain diseases like shothole fungus, as well as to recover from the effects of winter freezes, heavy crops, etc., and has, of course, given a larger tree that could bear a much heavier crop. Heavy pruning has had similar effects except that

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it generally tends to reduce the size of the tree, though in very thin, dry soil, heavy pruning has also increased the size of the tree.

Whitewashing Twigs to Retard Bud Growth. Another means of holding the buds dormant in winter is to keep all twigs and buds covered with whitewash. This keeps down the temperature of the buds during warm days by reflecting heat that would be absorbed by the dark colored pigments in the twigs. The effect of color on the temperature of the twigs has been worked out by Whitten[1] who found as great as eight degrees centigrade difference between air temperature and that of purple twigs in winter. He also found by carefully sectioning buds that when the twigs are kept whitewashed during the winter they do not push as rapidly during warm periods as do buds on twigs not whitewashed, and in some springs, though not in all springs, he was able to cause the blooming time to come somewhat later. However, it would not be expected that such a process would affect the blooming time as much as it affects the amount of starting during warm days in early winter, since at blooming time the buds are for a good share of the time exposed to an optimum temperature for growth or nearly so, and during warm days in winter the air temperature is below the optimum temperature for growth and often even below the minimum temperature. The temperature of these winter twigs may be raised by absorbing sunlight to at least above the minimum temperature for growth and in some cases to the optimum temperature. Thus those who have expected whitewashing to cause an appreciably later blooming in spring would naturally, in many cases, be disappointed. In sections, however, where much killing occurs in winter following the starting of buds by warm periods, it should be expected to give good results.

While prolonging the rest period by causing growth late in winter by the above method applies only to sections far enough south that there would be a considerable number of warm days before the rest period is entirely ended (sections like the southern half of Missouri and farther south), the benefits from whitewashing would be expected to be as great say in New York or Canada in March before the optimum temperature for growth is reached as in Missouri, unless the sunlight should be less direct and there should be less increase in twig temperature caused by the absorption of light by the dark color. However, the cost of whitewashing an acre of trees,

[1]Mo. Agr. Exp. Sta. Bul 38, 1897. (Bibl. No. 114.) Das Verhältnis der Farbe, etc., 1902 (Bibl. No. 116).

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(even assuming that the cost of one of the sprayings could be deducted since it could possibly be made to control either San Jose scale or peach leaf curl), would be in the neighborhood of $10 or $15 an acre, so before one would be justified in recommending such a method, it should be tried under orchard conditions for a long enough term of years to be sure that, say in twelve or fifteen years, enough peaches would be saved to justify the expense. There would unquestionably be some years when no benefit would be received in return for the whitewashing.

Killing Temperature of Peach Blossoms. A considerable amount of effort has been made to determine, under average conditions, the temperature at which the blossoms of peaches are killed. Unquestionably there is a considerable difference in the killing temperature of bloom in different years. The killing temperatures indicated by laboratory experiments will be given in the last part of this paper (Table 51.) Here the temperature at which bloom and young fruit has been killed in the orchard, and the condition which favor the smallest amount of killing will be discussed. During the spring of 1908 a freeze came on April 3rd when the temperature went to 24° F. at Columbia. Phenological notes taken that spring show that the first bloom on peach trees ranged from March 25th to March 30th, and full bloom ranged from April 5th to April 8th. The following table gives the percentage of bloom killed by this freeze:

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TABLE 46. SHOWING THE PERCENTAGE OF PEACH BLOOM KILLED ON THE BASAL END AND THE OUTER END OF TWIGS BY THE FREEZE OF APRIL 3, 1908, WHEN THE TEMPERATURE WENT TO 24° F.

Variety	Basal half of twigs		End half of twigs	
	Number Buds	Percentage Killed	Number Buds	Percentage Killed
Oldmixon	159	70.44	363	84.39
Oldmixon	426	11.03	598	41.97
Oldmixon	648	15.12	828	67.23
Elberta	374	19.78	603	44.92
Elberta	121	38.	205	69.2
Elberta	163	52.1	143	71.3
Crawford's Early	95	5.26	155	25.16
Ortiz	623	46.70	553	55.50
Sneed	503	14.25	492	65.44
Sneed	354	45.48	413	53.75
Sneed	251	23.1	186	50.5
Kalamazoo	579	32.29	529	54.23
Family Favorite	483	26.70	431	63.80
Family Favorite	256	22.22	192	66.14
Golden Gate	129	85.2	72	50.
Dewey Cling	106	14.1	109	73.4
Dewey Cling	171	12.2	111	17.1
Elberta Seedling	151	5.9	216	73.
Elberta Seedling	1850	11.	980	16.3
Connet	395	11.1	507	43.1
Total No. buds	8367	.....	8257	
Average percentage killed	.....	27.24	.....	53.68

Of course many of these blooms were not yet fully open and a much larger percentage of the flowers were fully open on the outer half of the twigs than on the basal half, as the following table will show:

TABLE 47. SHOWING PERCENTAGE OF BLOOM FULLY OPEN ON THE BASAL HALF AND THE OUTER HALF OF TWIGS BY APRIL 4, 1908

Variety	Basal Half		Outer half	
	Number Buds	Percentage Unopened	Number Buds	Percentage Unopened
Elberta Seedling	151	13.2	216	0.0
Elberta Seedling	1850	22.7	980	60.2
Connet	395	65.8	507	0.0
Sneed	174	16.0	252	0.4
Elberta	251	6.7	186	0.0

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Not nearly so large a percentage of unopened as open bloom was killed, as the following table will show:

TABLE 48. SHOWING PERCENTAGE OF OPEN AND UNOPENED BLOOM KILLED BY THE FREEZE OF APRIL 4, 1908

Variety	Buds Open		Buds Unopened	
	Number Buds	Percentage Killed	Number Buds	Percentage Killed
Oldmixon Free	253	69.9	30	36.6
Oldmixon Free	326	25.4	241	15.3
Elberta	347	24.4	85	7.1
Elberta	309	51.13	26	1.1

It will be seen that enough fully open bloom was uninjured at this temperature, 24° F., for a good crop if the tree had a heavy set of bloom. In fact a minimum thermometer that checked with those of the U. S. Weather Bureau registered a temperature in the Missouri Experiment Station orchard of 23° F., so in some years at least, peach bloom may be expected to withstand a temperature that low.

Those three tables bringing out the facts, that the bloom just before the petals open will withstand lower temperatures than fully open flowers; that the flowers open more rapidly toward the tips of the twigs, and that therefore flowers on the outer half of the twigs are less likely to survive a spring frost coming before the bloom is fully open, suggest that the tree should be pruned to a sufficiently open head that the leaves at the base of the twigs will not be shaded off before fruit buds are formed.

The greater hardiness of unopened buds is apparently not due to the protection of the petals. In freezing 59 unopened blooms to a temperature of -3° C., 11.8 per cent of the pistils were killed while of 50 unopened bloom with the petals and stamens removed, no pistils were killed.

On April 30, 1908, the temperature fell to 28° F. in Columbia. The calyx tube was just breaking from the young fruit. The following table gives the percentage of fruits killed at Columbia:

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TABLE 49. SHOWING PERCENTAGE OF YOUNG FRUITS FROM WHICH THE CALYX TUBE HAS JUST FALLEN KILLED BY A TEMPERATURE OF 28° F. APRIL 30, 1908

Variety	Number Fruits	Percentage Killed
Kalamazoo	749	26.3
Globe	279	22.5
Elberta	248	7.6
Elberta	301	.33
Elberta	300	5.6
Sneed	442	85.0
Alexander	411	76.8
Bonanza	454	5.94

It will be seen that large percentages of the fruits were killed only on early varieties like Sneed and Alexander that had reached a larger size. In the southern portion of Missouri where the fruit was larger, more was killed than at Columbia, though the temperature was higher, Koshkonong reporting 32° F. Of course in the lower land where most was killed, the temperature was naturally lower. It is apparently certain then that under average seasonal conditions, the older the fruit at the time of the freeze, the more easily it kills. It is possible that this would not be true in years when the bloom is pushed out very rapidly by exceptionally warm weather and when continuous cool weather follows the setting of the fruit. There is a rather general opinion that just when the calyx tube falls, the fruit is left more susceptible to cold because it has lost the insulation furnished by the calyx tube and requires time for adjustment. The fact that pistils of unopened flowers are not more easily killed when the external flower parts are removed, and the fact that buds of the peach were not more easily killed by freezing when the scales were entirely removed, would indicate that with the slow falling of the temperature that prevails under natural conditions, the insulation amounts to but little. It seems certainly true, from the experience of the season of 1908, that peach fruits are more tender a week or more after the calyx falls than say one day after it falls.

In examining, the fruits that were killed in the southern portion of the State in that year, in very many cases no injury was found to the flesh of the fruit, but the seeds were killed. Where the rate of fall of temperature is not too rapid under laboratory conditions, it is generally the seeds that are killed at the highest temperature. In fact from a large number of observations upon the results of

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freezing peaches in the laboratory, the tissue kills in the following order, beginning with the most tender: the veins surrounding the seed, the kernel, the flesh. When the peach has reached considerable size, the woody covering surrounding the kernel is most hardy.

The greater tenderness of the seed may be accounted for by the difference in sap density. Thus the freezing point depression for the seed kernels, when they are large enough to separate from the seed practically, was found to be 0.765° C., while that for the same fruit with the kernels excluded was 1.075°.

At Koshkonong and at Goodman in 1908, following the freeze of April 30th, the fruit was injured least on the young vigorously growing trees. Thus at Koshkonong in the orchard of Mr. W. C. Paynter, weak trees in various parts of the orchard and trees on rocky, uncultivated portions had all the fruit killed, while vigorous trees further down the hill where the temperature must have been lower, as well as further up the hill, bore a full crop. In the case of killing of buds in winter, whether they have been started into growth or not, and in the case of killing of blossoms in spring in the southern part of Missouri, in some years the Elberta is one of the most tender varieties. Yet where it is the young fruit that is killed, the Elberta seems to be one of the most hardy varieties, at least other varieties were killed to a larger extent than Elberta in that year. Early varieties where the fruit was large at the time of the freeze uniformly killed worse than later ones. In Missouri, at least, early varieties do not bloom earlier than late varieties like Salway. In fact in extreme South Missouri such early Chinese Cling varieties as Sneed, Victor and Carman, on account of their longer rest period actually bloom later than late varieties like Elberta or Crawford's Late or Heath Cling, yet from the time of pollination there is much more rapid growth of the young fruit of the early varieties. Thus when the calyx tube is dropping from the Elberta peaches, the young Sneed will be much larger.

As to means of handling the trees to avoid injury at blooming time or after fruit is set, there is not so much that can be done except that, as mentioned above, in the most southern portions of the peach belt it is possible, by increasing the vigor of the tree, to cause the blooming to be later and, therefore, the young fruit at any given time after the bloom falls, to be smaller when the frost might come. This, of course, would not apply further North. Vigorous, trees may also have their fruit killed to a smaller extent because, in their tendency to make wood growth, the fruit develops apparently more slowly, at least fruit is practically always later in ripening on vig-

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orous trees than on weakly growing trees. The whitewashing might also in some years have some effect on the amount of killing at blooming time, though it would probably have very slight effect on the amount of killing of young fruit after the bloom has fallen.

BREEDING VARIETIES HARDY UNDER SOUTH MISSOURI CONDITIONS

The most important ultimate means of reducing the amount of injury to both fruit buds and blossoms from low temperatures is by plant breeding. From what has been said above, for this southern region the varieties that would seem the most promising to use in breeding for hardiness would be some of the Chinese Cling group. With but few exceptions, the varieties of this group are more hardy than the average peaches, not only for this southern peach belt, but fortunately for the northern section also. Elberta, however, is a marked exception. It may be said further that the Chinese Cling comes nearly enough true to seed that it has been very useful in securing new varieties with size and shippping qualities desired. The quality, however, is rather low and the color poor so it must be crossed with something that will give color,—preferably yellow,—and quality. It seems highly probable that desirable hardy varieties of long rest period could be secured by crossing this strain with some high quality yellow fleshed peach like Fitzgerald, etc. However, the Elberta, the most promising example of this crossing, has certainly been a failure so far as hardiness is concerned. The Gold Drop and Lemon Free, being peaches of yellow flesh, fair quality and very hardy in bud for northern or southern climates, and rather hardy in wood, is promising, though so far we have been unable in the first cross to secure yellow flesh. The Hills Chili group may be among the hardiest varieties in both wood and bud for southern as well as for northern climates, but the quality is poor and resistance to rot so slight that it is a question whether they will be desirable for use in developing new hardy varieties for market conditions. Reference to Table 41 will show that the Green Twig varieties have as long rest periods as those of the Chinese Cling group. In addition to this they are hardy because their pale color reflects the sunlight instead of absorbing it;[1] yet their small size and indifferent quality, together with the fact that in all crosses with Purple Twig varieties they have taken the typical purple color, would seem

[1]J. C. Whitten, Mo. Agr. Exp. Sta. Bul. 38, 1897. (Bibl. No. 114). J. C. Whitten, Das Verhältnis der Farbe, etc., 1902. (Bibl. No. 117).

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to eliminate them as promising material for breeding hardy varieties of desirable quality.

Hardiness of Seedlings. With reference to the opinion some people have that seedlings are, for some unaccountable reason, more hardy than budded fruits, it may be said that in the season of 1911 the Missouri Experiment Station was able to secure the percentage of buds killed by two different freezes, one before any buds had swelled and one after some swelling, from seedlings of a number of common varieties, the most important being seedlings of Chinese Cling, General Lee, Elberta and Family Favorite of the Chinese Cling group, Lewis, Early Michigan and Hills Chili of the Hills Chili group; and of the Snow, one of the hardy Green Twig varieties. The following table gives the results:

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TABLE 50. SHOWING THE RELATIVE HARDINESS OF SEEDLINGS OF VARIOUS VARIETIES OF PEACH AS INDICATED BY PERCENTAGE OF BUDS KILLED BY A TEMPERATURE OF -8° F. JANUARY 5, 1911, AND BY A TEMPERATURE OF +6° F. FEB. 23, 1911.

Variety	Number of Buds	Percentage of Buds Killed Jan. 5, 1911	Percentage of Buds Killed Feb. 23, 1911	Percentage of Buds Alive After Both Freezes
Elberta	32	96.8	0.0	3.2
Elberta	89	79.8	7.8	12.4
Elberta	57	93.	5.4	1.6
Elberta	100	96.	4.	0.0
Elberta	133	66.9	9.	24.1
Elberta	36	100.	0.0	0.0
Elberta	107	80.4	12.2	7.4
Elberta	72	41.7	51.4	6.9
Elberta	103	77.6	16.6	5.8
Elberta	110	79.1	17.3	3.6
Elberta	60	85.	13.5	1.5
Elberta	28	96.4	0.0	3.6
Elberta	44	88.7	2.3	10.0
Elberta	60	66.3	20.0	13.7
Elberta	100	84.0	10.0	6.0
Elberta	38	78.9	2.6	18.5
Elberta	52	71.1	9.6	19.3
Elberta	54	98.2	1.8	0.0
Elberta	94	61.7	8.5	29.8
Elberta	9	100.0	0.0	0.0
Elberta	55	81.8	10.9	73.0
Elberta	26	88.4	11.6	0.0
Thurber	66	75.7	18.2	5.1
Thurber	63	46.0	33.4	20.6
Thurber	52	94.2	5.8	0.0
Thurber	90	75.5	18.1	6.4
Thurber	75	73.3	8.0	18.7
Thurber	136	69.8	13.3	16.9
Thurber	58	60.3	37.9	1.8
Thurber	80	41.2	20.0	38.8
Thurber	112	63.4	25.0	11.6
Thurber	101	75.2	9.9	14.9
Thurber	110	57.3	10.0	42.7
Thurber	32	71.9	6.2	21.9
Thurber	128	71.1	6.2	3.9
Thurber	56	57.1	42.1	10.8
Thurber	83	49.3	33.8	16.9
Thurber	122	31.9	51.7	16.4
Thurber	86	70.9	20.9	8.2
Thurber	160	28.1	28.8	43.1
Thurber	174	65.5	18.4	16.1
Thurber	143	68.5	25.2	6.3
Thurber	108	68.5	28.7	2.8
Thurber	63	82.5	12.7	4.8
Thurber	130	73.1	20.8	6.1
Thurber	150	67.3	22.0	10.7
Thurber	106	78.3	17.9	3.8

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Variety	Number of Buds	Percentage of Buds Killed Jan. 5, 1911	Percentage of Buds Killed Feb. 23, 1911	Percentage of Buds Alive After Both Freezes
Thurber	48	77.1	18.8	4.1
Thurber	118	84.7	13.6	1.7
Thurber	120	56.6	28.4	15.0
Thurber	78	97.4	2.6	0.0
Thurber	188	75.0	22.3	2.7
Thurber	133	92.5	6.0	1.5
Thurber	134	70.9	15.6	3.5
Thurber	152	82.9	15.1	2.0
Family Favorite	116	82.7	16.4	0.9
Family Favorite	106	75.4	14.2	10.4
Family Favorite	72	86.2	6.9	10.5
Family Favorite	86	74.4	15.1	10.5
Family Favorite	75	86.6	10.7	2.7
Family Favorite	108	59.3	32.4	8.3
Family Favorite	97	40.2	36.0	23.8
Family Favorite	107	74.8	15.9	9.3
Family Favorite	103	95.2	3.9	0.9
Family Favorite	100	94.0	3.0	3.0
Family Favorite	44	86.3	3.7	10.0
Family Favorite	185	48.1	44.3	7.6
Family Favorite	124	77.4	20.2	2.4
Family Favorite	98	79.5	19.4	1.1
Family Favorite	134	66.4	27.6	6.0
Family Favorite	122	72.9	24.6	2.5
Family Favorite	91	84.6	12.1	3.3
Family Favorite	132	72.7	25.8	1.5
Family Favorite	142	64.8	34.4	0.8
Family Favorite	145	79.3	20.0	0.7
Family Favorite	161	45.3	38.5	17.0
Family Favorite	133	73.7	24.8	1.5
Family Favorite	101	92.2	5.9	1.9
Family Favorite	154	70.1	28.5	1.4
Family Favorite	107	79.4	18.7	1.9
Family Favorite	124	86.3	12.9	0.8
Snow	281	33.8	51.6	14.6
Snow	170	32.4	61.7	5.9
Snow	290	34.5	58.6	6.9
Snow	145	40.7	43.4	15.9
Snow	265	35.6	57.8	6.6
Snow	231	33.7	57.8	8.5
Snow	224	40.0	58.0	2.0
Snow	232	43.5	54.3	2.2
Snow	298	25.1	61.4	13.5
Snow	226	28.7	60.0	11.3
Hills Chili	161	74.5	24.9	0.6
Hills Chili	118	83.0	16.1	0.8
Hills Chili	77	79.2	19.5	1.3
Hills Chili	133	77.4	19.6	3.0
Hills Chili	153	67.3	32.0	0.7
Hills Chili	105	50.4	44.7	14.9
Hills Chili	136	61.8	30.8	7.4
Hills Chili	136	55.1	40.4	4.5
Hills Chili	111	54.0	34.3	11.7
Hills Chili	135	57.0	31.1	11.9

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Variety	Number Buds	Percentage of Killed Jan. 5, 1911	Percentage of Killed Feb. 23, 1911	Percentage of Buds Alive After Both Freezes
Hills Chili.....	155	53.5	42.0	4.5
Hills Chili.....	154	55.2	36.3	8.5
Hills Chili.....	89	77.5	20.2	3.5
Hills Chili.....	233	49.8	45.5	4.7
Hills Chili.....	127	51.1	44.2	4.7
Early Michigan.....	153	91.0	9.0	0.0
Early Michigan.....	181	47.0	26.5	26.5
Early Michigan.....	94	57.4	16.0	26.6
Early Michigan.....	93	48.3	42.0	9.7
Early Michigan.....	81	39.5	12.3	48.2
Early Michigan.....	86	55.8	32.5	11.7
Early Michigan.....	112	62.5	12.5	25.0
Early Michigan.....	67	43.2	38.8	18.0
Early Michigan.....	184	27.2	47.3	25.5
Early Michigan.....	71	40.8	23.9	35.3
Early Michigan.....	57	33.3	54.4	12.3
Early Michigan.....	81	61.6	29.6	7.8
Early Michigan.....	74	37.8	35.1	27.1
Early Michigan.....	78	42.3	47.4	10.3
Early Michigan.....	75	90.6	8.0	8.6
Early Michigan.....	118	31.3	18.7	50.0
Early Michigan.....	63	50.8	39.7	9.5
Early Michigan.....	92	38.0	38.0	22.0
Early Michigan.....	94	57.0	38.3	4.7
Lewis.....	75	82.7	13.3	4.0
Lewis.....	111	65.8	30.6	3.6
Lewis.....	115	40.9	31.3	27.8
Lewis.....	181	43.0	45.3	11.7
Lewis.....	152	51.9	38.2	9.9
Lewis.....	132	73.5	19.7	6.8
Lewis.....	124	62.1	29.0	8.9
General Lee.....	231	37.2	7.4	55.4
General Lee.....	130	50.0	16.1	30.0
General Lee.....	229	65.9	22.2	11.9
General Lee.....	153	73.2	22.2	4.6
General Lee.....	139	35.2	30.9	23.9
General Lee.....	100	47.0	30.0	23.0
General Lee.....	88	58.0	38.6	3.4
General Lee.....	109	86.2	9.2	4.0
General Lee.....	108	71.3	19.4	9.3
General Lee.....	108	38.9	40.7	20.4
General Lee.....	112	44.7	15.3	40.0
General Lee.....	170	22.4	40.0	37.6
General Lee.....	131	60.3	21.4	18.3
General Lee.....	115	63.4	15.7	20.9
General Lee.....	105	32.4	10.5	57.1
General Lee.....	101	39.6	17.8	42.6
General Lee.....	114	77.2	20.2	2.6
General Lee.....	109	75.3	12.8	11.9
General Lee.....	100	78.0	7.0	15.0
General Lee.....	102	50.0	28.5	21.5
General Lee.....	131	77.1	22.9	0.0
Chinese Cling.....	154	63.6	35.0	1.4
Chinese Cling.....	149	62.4	14.8	22.8

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Variety	Number Buds	Percentage of Killed Jan. 5, 1911	Percentage of Killed Feb. 23, 1911	Percentage of Buds Alive After both Freezes
Chinese Cling.....	118	37.2	28.1	34.7
Chinese Cling.....	204	33.3	20.0	46.7
Chinese Cling.....	64	84.4	12.5	3.1
Chinese Cling.....	123	54.5	32.5	13.0
Chinese Cling.....	121	39.6	38.1	21.3
Chinese Cling.....	176	34.6	28.4	37.0
Chinese Cling.....	219	34.2	13.2	51.6
Elberta, average.....				10.92
Thurber, average.....				11.5
Family Favorite, average.....				5.4
Snow, average.....				8.7
Hills Chili, average.....				8.3
Early Michigan, average.....				19.9
Lewis. average.....				10.4
General Lee, average.....				21.6
Chinese Cling, average.....				23.5

It should be said that the varieties of the Hills Chili group are usually less vigorous growing trees, and since these seedlings were very closely planted, trees of the Hills Chili group would suffer more from shade in summer and thus have a larger percentage of buds not fully developed that are killed during even a mild winter. It will be seen that as a general thing those varieties which are most hardy have given seedlings that are also most hardy. By referring to this table and to Table 39 it will be seen also that these seedlings were generally not more hardy than the parents, though of course in the case of Elberta—a cross between the rather hardy Chinese Cling and Crawford's Early, and tender like Crawford's Early—some of the seedlings would tend to be more hardy than Elberta.

KILLING OF APPLES

Killing of the Roots. Killing of roots was not discussed for peaches because so far as can be learned, the killing of peach roots does not vary greatly from the killing of apple or of other fruit-tree roots. The killing of apple roots is probably a more common phenomenon than the killing of peach roots because peach roots are not so much more tender than apple roots as peach wood or peach fruit buds are more tender than apple wood or apple fruit buds. On account of the greater tenderness of wood and fruit buds, the peach tree

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is not grown commercially in as severe climates as those in which apples are sometimes grown, yet killing of roots of peach trees is observed in fairly important commercial peach districts.[1]

Macoun[2] reports that killing of apple roots is fairly common in the northern portion of the apple growing section and says that the root killing they have had in Canada has been when apples were worked on seedlings of southern forms, and that since the crab has been used as stock, the killing has been much less.

Emerson[3] at the Nebraska Experiment Station planted apple trees in boxes two feet square and eighteen inches deep, each box having twenty-five young roots. The boxes were left out of doors about the middle of December, the soil having different percentages of moisture. The trees were examined on February 25. In an unprotected box containing 10.4 per cent of moisture, twenty roots were killed, only five remaining uninjured. Another unprotected box containing 15.2 per cent of moisture had nineteen killed and six injured. In a box containing 19.8 per cent of moisture, three were killed, ten injured and twelve uninjured. A box covered with straw mulch and containing 16 per cent of moisture had none of the roots killed, and only seven injured. A box covered occasionally with snow and containing 15.8 per cent of moisture had seven dead, eight injured and ten uninjured. No roots were injured in a box stored in a cool, dry cave, though it contained but 10 per cent of moisture. It is shown from this experiment that the low moisture content in itself did not do the harm, but the low moisture associated with low temperature. It does not seem, however, that one is justified in concluding, as Macoun apparently does, that plants at any given temperature kill worse in media with as small as 10 per cent of moisture than in media with 20 per cent of moisture. From experience here it would seem likely that the reverse is true. Careful temperature records would probably indicate that the temperature was lower in the boxes containing the smaller moisture content, and a dry soil will freeze more deeply than will a moist soil. Thus a mulch or sod or cover crop that tends to prevent evaporation from the soil will prevent its freezing so deeply and thus prevent injury to the roots by keeping the temperature higher.

Craig[4] studied root killing in Iowa for the winter of 1898-99. The injury was worst in light dry soils not protected by a cover crop

[1]Stone, Mass. (Hatch) Exp. Sta. Rpt. 1911, p. 66. (Bibl. No. 107). Green, W. J. and Ballou, F. H., Ohio Agr. Exp. Sta. Bul. 157, 1894. (Bibl. No. 48).
[2]Canada Exp. Farms, Rpt. 1907-8, pp. 110-16. (Bibl. No. 68).
[3]Nebraska Agr. Exp. Sta. Bul. 79, 1903. (Bibl. No. 33).
[4]Iowa Agr. Exp. Sta. Bul. 44, 1900. (Bibl. No. 25).

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In case of light soils like the "loess" he found that deep planting helps to reduce the danger from root killing. Such soils would of course freeze more deeply and the temperature of the roots would be lower. He had little opportunity to observe directly the difference in hardiness of different stocks, except in one case where Shield Crab was used as stock, three-year nursery trees of Jonathan, Whitney, Grimes and Willow came through the winter in good condition, though nursery trees generally were badly injured. In examining injured trees he found that the stock at the union of stock and scion was often killed while immediately adjoining scion tissue was uninjured. He found scion roots to be more resistant than stock roots. This is in accordance with experience here in freezing roots in the laboratory. We have also found that the root system becomes more tender as it gets further from the crown. Emerson and others have observed that roots of trees are killed at a much higher temperature than are other tissues. This has been our experience. Results with freezing roots of orchard trees will be found in Tables 30, 31, 32, 33, and 34.

In case of one-year-old roots of the French crab used as stock by most of the nurserymen, about 5° C. to -8° C. is as low a temperature as they can be depended upon to withstand with no injury. Of 90 Jonathan apple grafts with French crab stock frozen to a temperature of -9° C. or lower and then planted in soil in the greenhouse, none lived. Four out of ten lived after being frozen to a temperature of -8° C.; three out of ten after a temperature of -7° C.; six out of ten after a temperature of -6° C., and practically all grew after a temperature of -5° C. These roots had not been exposed to a high temperature during the winter preceding the freezing, which was done from March 2, to March 18, 1911.

According to Craig and also Stone, when the roots are frozen, the results do not show to the inexperienced at once in spring. The trees will often bloom and usually the leaves will partially expand before the injury begins to show clearly. If only a part of the root system is killed, only certain connected branches will show the effects of the root killing.

Injury to Apple Wood. Macoun[1] lists a number of forms of winter injury to wood of fruit trees, especially the apple, as follows:

Bark splitting, which he says usually occurs when growth has continued late in autumn and an early summer has prevented the soil from freezing.

[1]Canada Exp. Farms Rpt. 1907-8, pp. 110-16. (Bibl. No. 68).

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Trunk-splitting, sun scald, a killing of the bark on the south or southwest side of the tree which he reports very serious in northern and eastern Ontario and in the Province of Quebec.

Killing-back which he says results from inherent tenderness of the variety, or from insufficient maturity of the wood.

Crotch-injury, a killing of the cortex and perhaps other tissue in crotches of the limbs. Macoun, Morse, and Grossenbacher have studied this form of injury, and have found it to follow severe winters. Macoun and Morse attribute this injury to the lodging of ice in the crotch, while Grossenbacher attributes it to tearing caused by tensions developed by the shrinking of the tissue during its frozen condition. Grossenbacher observed this form of injury most commonly in the crotch formed by vigorously growing secondary branches.

Black-heart, a condition which follows the killing of the sap wood when the cambium is left alive to form new wood outside the killed area. It is troublesome to nurserymen in northern sections.

Trunk-injury or body injury, a killing of the older parts of the tree above the snow line. Macoun thinks this injury may be due to loss of water while the tree is frozen. Grossenbacher[1] describes a similar injury as Crown Rot, and attributes it to tearing from tension in the tree when frozen, and to a loss of water while frozen. He seemed to find it worse on the side of the tree trunk next to the prevailing winds.

The experience of the Missouri Experiment Station where the wood of the body of the tree, especially at the base of the tree trunk and at the crotches, becomes hardy more slowly in autumn in some years at least than does the tissue of the secondary branches or even the twigs (see Table 24) may have some bearing in connection with some of the above forms of injury, especially crotch injury and body injury, or crown rot. It seems possible that those forms of injury are merely direct freezing to death and that such injuries are more commonly found at the base of the trunk, or at the crotch, because on such years these are the most tender points.

Grossenbacher thawed the bark of a tree with warm water and worked the tree backward and forward when the temperature was -26° C. on January 8. In March practically all of the bark was found to be dead, showing apparently the characteristic browning of direct freezing to death. It would seem doubtful if this condition of complete death of the tissue with the browning would be found so soon

[1]New York (Geneva) Agr. Exp. Sta. Tech. Bul. 23, 1912. (Bibl. No. 50). New York (Geneva) Agr. Exp. Sta. Tech. Bul. 12, 1909. (Bibl. No. 51).

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if it were merely a mechanical injury such as tearing loose of the bark. In this case it seems highly probable that the killing resulted from rapid temperature fall. The thawed tissue would fall to the temperature from which it was thawed, very rapidly, and from experience here with rapid freezing (see Table 20) it would seem probable that this tissue was killed by direct freezing the same as buds would be on account of this rapid lowering of the temperature.

Sun scald is generally thought to result from the effects of the heat gathered by the dark tissue that is in the direct sunlight. Since Macoun reports sun scald as being serious in climates as far north as the Province of Quebec, it would seem doubtful if there would be enough heat gathered on the sunny side of a tree trunk in winter there to raise the tissue to a temperature high enough for growth to take place. From experience here where the rate of temperature fall makes such a large difference in the killing, it seems highly probable that, at least in northern sections, sun scald may not result from the tissues starting into growth, but it more likely results from the temperature being raised to near the freezing point on sunny days during cold weather and dropping very rapidly to the temperature of the air as soon as the sun is off that part of the tree trunk.

As to varieties most hardy in wood under extreme conditions, Macoun[1] reports an examination of about 700 varieties in which he found that summer and fall apples were generally more hardy in wood than late winter varieties, probably because their wood reaches a condition of maturity earlier. In his experience some of the most hardy varieties were: Canada Baldwin, Winter Rose, Ontario, Stockton and McIntosh, all apples of northern origin. Oldenburg was cited by him as being one of the hardiest in wood.

The group of apples which Hedrick lists as hardy for the coldest part of the state of New York are: of summer apples, Yellow Transparent, Tetofsky, Oldenburg, Red Astrachan, and Lowland Raspberry; and of fall and winter apples, Wealthy, Hibernal, and Fameuse groups. Among the varieties that would not withstand the cold in the northern districts were: of summer apples, Early Harvest; and of fall and winter apples, Baldwin, Black Gilliflower, Jonathan, Rome, Winesap, and in the very coldest region, even the Northern Spy group.

The Killing of Apple Buds. The buds of apples will withstand much lower temperature than will the buds of peaches or of even plums and cherries, and Macoun reports the killing of buds of apples

[1]Canada Exp. Frt. Farms, Rpt. 1906, pp. 291-92. (Bibl. No. 70).

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even in a very dormant condition. Whipple[1] describes a considerable amount of killing he has observed in Montana. When apple buds are killed it is not necessarily true that all parts of the buds are killed; generally only the flower parts, so that the buds will open in the spring into a whorl of leaves and for this reason the fact that any killing occurred may not be observed. He found also that in some cases not even all the flower parts were killed, but on opening, various malformations were to be observed; thus in some the pistils were entirely absent and in some both stamens and pistils. In some cases seedless apples were developed from such flowers.

Under Missouri conditions, especially in the Ozark region in the thinner soil, we have observed a considerable amount of killing by the freezes of December 9 and 29, 1909, when the temperature went to -5° F. and -8° F. respectively. During the same year buds of the York Imperial, as well as Jonathan, that had been largely killed by the same freeze, were sent in from points in Illinois. In most cases, however, the entire bud was killed. The Jonathan is the one variety in which a considerable amount of such injury has been observed. In low places where the cold air may settle and result in a very low temperature, all of the Jonathan buds were killed, even on healthy trees. However, in the Missouri Experiment Station orchard following a temperature of -20° F. on January 7, 1912 not a large percentage of the buds were killed. Of 200 Jonathan buds counted, 38 per cent were found to have been killed, and of the same number of Ben Davis buds counted, none were found dead. Of course some buds in which the flower parts were injured may have been overlooked. However, a good crop was secured in that year. In this orchard the trees had been kept in a healthy condition, while practically all the orchards observed, and all the orchards from which twigs were sent, were neglected orchards where the trees had made a weak growth the summer before and had set their fruit buds very early, probably pushing them too far early in the season before this freeze.

Killing of Apple Bloom. Killing of the flowers is a common form of injury to apples resulting from low temperatures, at least under Missouri conditions. An effort was made to determine what is approximately the killing temperature of the full bloom of apples. The following table gives the results of freezings with apple, peach and other fruits, just before the bloom opens, in full bloom, just after the bloom falls, and when the fruit is as large as it has been when it

[1]Montana Agr. Exp. Sta. Bul. 91, 1912. (Bibl. No. 113).

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was killed in Missouri. The material was generally kept at the minimum for thirty minutes. The temperature fall was as slow as it could be made with the freezer used.

TABLE 51. GIVING RESULTS OF ARTIFICIAL FREEZING OF FRUIT BLOSSOMS

Material	Condition of Flowers When Frozen	Date	Temperature	Number Blossoms	Percentage Killed
Apple, Ben Davis.....	Not fully open	Apr. 14,'11	27.5	87	31.0
Peach, Elberta Seedling	Fully open....	Apr. 14,'11	27.5	66	6.1
Pear, Kieffer.....	Fully open.....	Apr. 14,'11	27.5	40	0.0
Plum, Wild Goose.....	Fully open....	Apr. 14,'11	27.5	60	0.0
Cherry.....	Not fully open	Apr. 14,'11	27.5	97	50.5
Apple, Stannard.....	Fully open....	Apr. 15,'11	27.5	45	40.0
Peach, Elberta Seedling	Fully open....	Apr. 15,'11	27.5	87	60.9
Pear, Kieffer.....	Fully open....	Apr. 15,'11	27.5	70	32.8
Plum, Wild Goose.....	Fully open....	Apr. 15,'11	27.5	50	0.0
Cherry.....	Fully open....	Apr. 15,'11	27.5	91	58.2
Apple, Ben Davis.....	Fully open....	Apr. 15,'11	27.5	61	22.9
Peach, Rareripe.....	Petals falling..	Apr. 28,'13	26.6	86	2.5
Apple, Jonathan.....	Not fully open	Apr. 28,'13	26.6	100	8.0
Apple, Jonathan.....	Fully open....	Apr. 28,'13	26.6	78	56.4
Apple, Jonathan.....	Petals falling..	Apr. 28,'13	26.6	120	18.0
Cherry, English Morello	Fully open....	Apr. 28,'13	26.6	130	48.5
Cherry, English Morello	Petals falling..	Apr. 28,'13	26.6	107	67.1
Apple, Rome Beauty...	Fully open....	May 1,'13	24.8	83	40.9
Peach, Hiley.....	Young fruit...	May 1,'13	24.8	76	1.3
Cherry, Montmorency..	Fully open....	May 1,'13	24.8	40	52.5
Apple, Jonathan.....	Young fruit....	May 10,'13	26.6	53	71.7
Peach, Early Bernard..	Young fruit...	May 10,'13	26.6	74	94.6
Peach, Gold Drop.....	Young fruit...	May 10,'13	26.6	65	75.4
Cherry, Dyehouse.....	Young fruit...	May 10,'13	26.6	44	70.4
Plum, Wild Goose.....	Young fruit...	May 10,'13	26.6	93	16.1
Pear, Kieffer.....	Young fruit...	May 10,'13	26.6	32	78.1
Apple, Jonathan.....	Young fruit...	May 17,'13	24.8	19	68.4
Peach, Lewis.....	Young fruit...	May 17,'13	24.8	23	43.5
Cherry, Dyehouse.....	Young fruit...	May 17,'13	24.8	15	66.6

These artificial freezings are not accurate in determining the minimum temperature which the bloom, etc., will withstand, since it is not possible to duplicate in the freezer the rate of temperature fall, etc., outside, nor is it possible to make two freezings exactly duplicate each other. However, it is safe to conclude from this table that the unopened flowers are slightly more hardy than the fully open ones, and that the fruits are slightly more tender than the flowers. The difference with apples, judging from these freezings and from our observations on freezings outside, is not so great as with peaches.

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These results indicate that peaches, either in blossom or young fruit, will withstand slightly lower temperatures than will apples. The greater hardiness of the peach bloom is probably under-emphasized in this table. In the year 1911 the Elberta Seedling peach tree came into full bloom on April 12, while the Ben Davis apple came into full bloom on April 18 to 22, and the Stannard apple on April 20. It is almost certain then that the peach blossoms listed as fully open were older than the apple blossoms so listed and had been pollinated and had started rapid growth, while the apples had not. In 1913 the Early Bernard peach came into full bloom on April 11, and the Hiley and Rareripe on April 16-17, while Jonathan apple came into full bloom April 24, and the Rome on April 29. Here then the young fruits of the peach were older than were the young apple fruits used. From results of freezes as seen in the orchard, we are convinced that at times, at least, bloom and young fruit of peaches will withstand lower temperatures than will apple bloom or young fruit.

The freeze of April 30, 1908, when the temperature in Columbia went to 28° F., killed only the percentages of young peach fruit shown in Table 49, and an excellent crop was secured from the peach trees the summer of 1908, while practically all the apple fruit was killed; yet the peach fruit had been set longer. The peach trees in that year came into full bloom from April 5 to 7, while varieties of apples like Ben Davis, Jonathan and Grimes, fruit of which was all killed in this orchard, came into bloom from April 15 to 17. The Ingram apple in that year came into full bloom on April 22 to 24 and had some bloom left when the freeze came. It had a fair crop of apples left, and the Ralls, which came into full bloom from April 23 to 25 and had a considerable amount of open bloom when the freeze came, had very few fruits or blooms killed. If peach blooms or fruits are more often killed in spring than that of the apple, it is because peaches usually bloom considerably earlier.

In avoiding a loss from low temperatures in spring, the most important factor is late blooming. Some varieties like Ingram and Ralls bloom so much later than ordinary varieties that, except in very few sections of the State, they are practically never killed by spring frosts. Other varieties that apparently are seldom killed under Missouri conditions are Benoni and Mother, according to information furnished by Mr. F. W. Faurot, formerly of the Missouri State Fruit Experiment Station at Mountain Grove, Missouri. Growers in southern Illinois also report Benoni as being rather safe from frosts. Rome Beauty on many years blooms enough later than other varieties to escape injury. Whether in the southern portion

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of the apple growing region this blooming in spring can be caused to be delayed by prolonging the rest period, as in the case of peaches, has not been studied. Mr. Faurot however, tells of an instance in which cultivated trees were three days later in coming into bloom than uncultivated trees of the same variety, with like treatment in every other way. The fact that the buds on well kept Jonathan orchards on good soil were not killed to such a large extent by the freezes of December, 1909, as were the buds on trees in neglected orchards that had gone dormant much earlier the season before, seems to indicate that the buds in early winter, at least, are kept dormant later by keeping the tree vigorous.

An opinion is held by some fruit growers and horticulturists that if the tree is in a vigorous condition the bloom or young fruit will withstand lower temperatures because of the healthier condition of the bloom or young fruit. Three Gano apple trees on the Station grounds have been left unsprayed and uncultivated and are in a weak condition, one of them in a very weak condition. At times bloom or young fruit from these have been frozen along with bloom or young fruit from well kept Gano trees. These weak trees and the vigorous trees in Columbia bloomed at the same time. The following table gives the results:

TABLE 52. SHOWING RELATIVE HARDINESS OF BLOOM AND FRUIT FROM WEAK AND FROM VIGOROUS GANO APPLE TREES

Material	Date	Temperature	Number Frozen	Percentage Killed
Vigorous sprayed tree	May 1,'12	−3	28	71.4
Weak unsprayed tree	May 1,'12	−3	16	87.5
Vigorous sprayed tree	May 1,'12	−5	50	100.0
Weak unsprayed tree	May 1,'12	−5	40	95.0
Vigorous sprayed tree	May 4,'12	−2	52	21.1
Weak unsprayed tree	May 4,'12	−2	56	19.7
Very weak unsprayed tree	May 4,'12	−2	46	32.6
Vigorous sprayed tree (fruit ⅜ in. in dia.)	May 18,'12	−4	98	81.9
Very weak unsprayed tree (fruit ⅛ in. in dia.)	May 18,'12	−4	79	83.5

It will thus be seen that there is no constant difference in the hardiness of the fruit from the weak and the vigorous tree. Casual observers may mistake loss of crop from cool weather at blooming

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time, poor pollination, or other reasons, for injury from freezing. It is certainly true that a poor set of fruit is often attributed to frosts when a careful observation following the frost would have shown that no bloom or fruits were killed.

KILLING OF CHERRIES AND PLUMS

Killing of various tissues of other fruits has not been extensively studied at this Station, except as described in the early part of this paper. As to killing of buds, Macoun[1] states that under Canadian conditions, especially in the Province of Quebec when away from the protection of a body of open water, European and Japanese plums, and cherries are injured more or less every winter. He lists Mount Royal and Raynes as new varieties very hardy in bud. Japanese plum fruit buds have killed in Missouri only after cold periods following sufficient warm weather to start them into growth.

In case of killing by spring freezes, the Wild Goose plum is very resistant to low temperature as will be seen by reference to Table 51. On April 24, 1910, when all other fruits in the Station orchard, except Ingram and Ralls apples which were just in bloom, were killed by a temperature of 27° F. Wild Goose plums were uninjured though they had reached a diameter of 3-16 of an inch. We are not prepared to say, however, that the young fruit of the Wild Goose plum is not more hardy than the bloom.

In the case of cherries observations have been made on the killing of the Early Richmond cherry buds to a large extent, and other varieties to a small extent, especially by cold periods following warm weather. On January 7, 1912, the following percentages of fully dormant cherry buds were killed by a temperature of -20° F.: Early Richmond, of 200 buds counted, 52.5 per cent were killed; Montmorency, of 150 buds counted, 12 per cent were killed; Dyehouse, of 175 buds counted, 11.4 per cent were killed.

Macoun holds that fruit buds of cherry, peach and Japanese and European plum kill more easily than do the buds of the apple and the pear because they have less protection from evaporation. Goff[2] found a larger percentage of cherry buds to survive a temperature of -27½° F. in Wisconsin in February, 1899, in the central portion of the tree than on the ends of the outer branches. He concludes that this results because those near the center of the tree were partially protected from drying winds. It does not seem probable

[1]Canada Exp. Farms, Rpt. 1907-8, pp. 110-16. (Bibl. No. 68).
[2]16th An. Rpt. Wis. Agr. Exp. Sta. pp. 283-88, 1899. (Bibl. No. 46).

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that evaporation affects the killing of buds unless it be indirectly by the drying out of the twigs. Cherry buds are more tender than apple buds when exposed to sudden freezes like that at Columbia, Missouri, January 7, 1912. They are also more tender when frozen in the laboratory under conditions such that buds with the scales removed kill no worse than normal buds. Evaporation could certainly play no part there. The buds at the ends of peach twigs are generally more tender than those near the base of the twigs or on spurs along the branches, but buds on the ends of twigs down in the tree are as tender as any. It is probable that the buds that Goff found more hardy toward the center of the tree were formed earlier in summer and were more mature when the freeze came.

One of the most promising means of avoiding loss from low temperatures is by orchard heating, burning oil, coal or other material. In some sections this practice is followed profitably, and it is recommended in others. This subject is being studied at the Missouri Experiment Station by Dr. W. L. Howard. In the work here reported, no data has been gathered that is of value in a study of orchard heating, unless the results with freezing bloom and young fruit may be of some value in determining the temperature at which heaters should be lighted. Data on the killing temperature of the bloom of fruit to be protected is very essential in orchard heating, otherwise the heaters will too often be lighted and large expense incurred when it is unnecessary, or perhaps sometimes they may not be lighted when it is necessary, and loss will be incurred. A study of orchard heating, like a study of the value of whitewashing as a means of preventing killing from cold, should be carried on through a large number of years since heating involves an expense certainly not less than an average of $10 or $12 per acre a year, besides a large initial investment, and the mere fact that the crop can be saved by heating is no positive indication that in a period of twelve or fifteen years enough crops will be saved, in spite of accidents inherent in the method of heating, to pay for the cost of heating.

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RESEARCH BULLETIN NO. 9.

UNIVERSITY OF MISSOURI

COLLEGE OF AGRICULTURE

AGRICULTURAL EXPERIMENT STATION

CAROTIN—THE PRINCIPAL NATURAL YELLOW PIGMENT OF MILK FAT—PART I

Review of the Literature Concerning the Yellow Plant and Animal Pigments

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COLUMBIA, MISSOURI
April, 1914

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FOREWORD.

The investigational work upon which this bulletin is based was conducted in co-operation with the Dairy Division of the United States Department of Agriculture. The bulletin herewith presented is Part I of a series of four bulletins reporting a detailed investigation of carotin, the principal natural yellow pigment of milk fat—its sources, its relation to feed consumed and milk produced, factors which are influenced by it as well as the factors by which it is influenced. Part I is prefatory in nature; the investigation proper is reported in parts II, III, and IV.

F. B. MUMFORD,
Director.

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CAROTIN—THE PRINCIPAL NATURAL YELLOW PIGMENT OF MILK FAT.

Its Relations to Plant Carotin and the Carotin of the Blood Serum, Body Fat and Corpus Luteum.

LEROY S. PALMER AND C. H. ECKLES.

The investigations dealing with the natural yellow pigment of milk fat will appear as a series of four bulletins as follows:

Part. I. A Review of the Literature Concerning the Yellow Plant and Animal Pigments. Missouri Agricultural Experiment Station Research Bulletin No. 9.

Part II. The Chemical and Physiological Relation of the Pigments of Milk Fat to the Carotin and Xanthophylls of Green Plants. Missouri Agricultural Experiment Station Research Bulletin No. 10.

Part. III. The Pigments of the Body Fat, Corpus Luteum and Skin Secretions of the Cow. Missouri Agricultural Experiment Station Research Bulletin No. 11.

Part IV. (A). The Yellow Lipochrome of Blood Serum. (B.) The Fate of Plant Carotin and Xanthophylls During Digestion. (C). The Pigments of Human Milk Fat. Missouri Agricultural Experiment Station Research Bulletin No. 12.

The present paper is the first of the series. As indicated it will be confined entirely to a review of the extensive literature in regard to the yellow plant and animal pigments.

Part II will be a report of the chemical identification of the milk fat pigment. It will also include a number of investigations showing the relation between the amount of pigment in the milk fat and the character of the ration and the breed of the cow.

Part III will consist of the data showing the chemical identification of the pigments mentioned. Data will also be presented showing the relation between the color of the body fat and the character of the

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ration and the breed of the cow. A brief experiment will also be reported showing the absence of these pigments in the body of a newborn Jersey calf.

Part IV (A) will report the chemical identification of the blood serum pigment. It will show how blood carries the pigment and what effect the character of the ration has upon the amount of pigment carried by the blood and the amount secreted in the milk at the same time. A brief study of the cause of the high color of colostrum milk will also be reported. (B) This will consist of the report of a few investigations relative to the fate of the carotin and xanthophylls of plants during their passage through the cow's body. (C) The experiments reported here will show the character of the pigments of human milk fat.

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A REVIEW OF THE LITERATURE CONCERNING THE YELLOW PLANT AND ANIMAL PIGMENTS.

It has been the custom for generations to judge the quality of dairy products to a large extent by their yellow color. This has been carried to such an extent that the manufacturer of butter, whether it be on a large or small scale, finds it impossible to market butter that does not have a standard yellow color. The consumer of milk or cream as a rule looks upon a yellow color as indicating the richness and quality of the product. Although it is well known that the color has no relation to the food value of milk or cream, the popular prejudice is so strong that the producer of market milk has to take it into account and try to supply a product with as much natural yellow color as possible.

During part of the year, namely during the spring and early summer and usually also in the early fall, the fresh green feeds which the cows receive give the shade of yellow to the milk fat which the consumer demands. During the winter months, or in summer if the pastures become dry, this yellow color is wholly or in part absent from the milk fat, and the butter manufacturer is then forced to color the butter artificially, in order to maintain the required standard.

It is generally accepted as a fact that the breed of the cow has a pronounced relation to the color of the milk fat and that the Guernsey and Jersey breeds rank first in this respect. The breeders of this class of cattle have emphasized this characteristic as one of the strong points of their respective breeds. This characteristic of Guernsey and Jersey breeds, as compared with the Holstein and Ayrshire breeds, has been generally attributed to physiological differences. According to this view, Guernsey and Jersey cattle are able to produce a higher colored fat due to some inherent quality, just as they are able to produce a higher percentage of fat in their milk. It is a well-known fact that the skin and the secretions of the skin of Guernsey and Jersey cattle have a higher yellow color than other breeds, and this characteristic is looked upon by cattle breeders as an indication of the ability of animals of these breeds to produce highly colored milk fat.

The body fat of Guernsey and Jersey cattle is also characterized by a high yellow color and for this reason beef from these animals is often looked upon with disfavor by the butcher and the consumer.

That the yellow color of butter has a relation to its market value is shown by the fact that "color" has a place on the standard butter score cards with a value of fifteen out of one hundred points. The

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oleomargarin manufacturers have also recognized the value of color and, so far as the law has permitted, have made a practice of coloring oleomargarin in imitation of butter. When the law placed a tax on artificially colored oleomargarin, or in some cases prohibited it entirely, the manufacturers began using only the highest colored beef fats that could be bought or mixed the oleomargarin with butter having a high natural color, in order to produce the color they sought.

The Pigment of the Butter Fat as a Factor in the Coloration of Milk.

The more or less yellow color of cows' milk which is especially evident in the cream and butter has not been attributed in all cases to the same pigment. On the one hand a few authors have stated that the pigment of butter is manifested in the familiar yellow color of milk whey. This view originated with Blyth [1] who called the whey pigment lactochrome and the view has found its way into a number of texts. On the other hand a larger number of authors have ignored the whey pigment and considered the lipochrome-like pigment of the milk fat to be the only factor causing the yellow color of cream and butter.

The investigations which were carried on in this laboratory have been the first to point out that the whey pigment and the butter fat pigment are not identical but are distinct substances; and that both are of importance in causing the yellow color of milk. The pigment of the butterfat is the more important of the two, however. The pigment of the whey is of secondary importance, and is of an entirely different nature. Its probable identity with urochrome, the specific urinary pigment, has recently been shown by one of us.[2]

Object of the Present Investigations.

The present investigations were undertaken primarily to study the chemical nature of the yellow butterfat pigment and to classify it from a scientific standpoint. At the same time information was gathered with the hope of ascertaining to what extent the generally accepted views concerning the color of milk fat are correct in order to establish a scientific basis for the subject which would be of value to those interested in the handling of dairy products in a commercial way.

In the principal part of the investigation it was sought; (1) to show the chemical and if possible the physiological relation of the butter fat pigment to similar animal pigments such as the

1. A. W. Blyth, "Foods. Their Composition and Analysis" Text, 4th Edition 1896, p. 239.

2. Lactochrome: The Yellow Pigment of Milk Whey, etc., by Leroy S. Palmer and Leslie H. Cooledge. Missouri Agricultural Experiment Station Research Bulletin No. 13;; Jour. Biol. Chem. XVII, p. 251 (1914).

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corpus luteum pigment, the body fat pigment, and the blood serum pigment; and (2) to show the chemical and physiological relation of the butterfat pigment to the carotin and xanthophylls of green plants.

In the secondary part of the investigation it was sought to study the influence of certain factors which have both practical and scientific bearing upon the color of the butterfat, among which are the breed of the animal and the character of the ration, the latter in connection with the chemical and physiological studies indicated above.

THE PIGMENTS OF PLANT ORIGIN.

The earliest researches on plant pigments dealt with the green pigments. Caventon first called them chlorophyll in 1817. His work, however, was preceded by the pioneers in this field, among which the names of Grew, whose work is dated 1682, and Rouelle, Meyer, Fourcroy, Berthellot, Senebier, Proust and Vanquelin are of historical interest.

The Carotins.

The Pigment of the Carrot. The yellow pigment of the cultivated carrot (Daucus Carota) has long been of interest to botanists and chemists, the investigations of this body having extended over almost one hundred years.

Wachenroder [1] was the first investigator of the carrot pigment. He isolated it and called it Karotin. The work of Vanquelin and Bouchardat [2] soon followed and a little later Zeise [3] took up the study. He obtained the first crystals and assigned to them the chemical formula $C_5 H_{10}$ or 10 ($C_5 H_8$).

Husemann [4] was the next investigator. He found six per cent of oxygen in his pure preparation and gave the pigment the formula $C_{18} H_{24} O$. A secondary pigment which he thought always accompanied the carotin in small amounts, he named hydrocarotin and gave it the formula $C_{18} H_{30} O$.

It is to Arnaud [5] however that we are indebted for the first thorough research in regard to the carrot pigment carotin. The crystals which he obtained were flat, rhombic-shaped crystals, red orange by transmitted light, and greenish blue by reflected light. They melted at 168° C. He showed beyond a doubt that the pigment was simply

1. Dissertatio de Anthelminticis Göttingen 1826; also Geigers Magaz. Pharm. 33 p. 144 (1831); also Berzelius Jahresber. 12 p. 277 (1833).
2. Schweizg. Jour. Chem. 58, p. 95 (1830).
3. Lieb. Ann. 62 p. 380 (1847); Annal. Chem. Phys. (3) 20, p. 125 (1847).
4. Lieb. Annal. 117 p. 200 (1860).
5. Compt. Rend. 102 p. 1119 (1886), p. 1319 (1887); Jour. Pharm. Chim. 14 p. 149 (1886).

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an unsaturated hydrocarbon. He gave it the formula C_{26} H_{38} and the iodine derivative the formula C_{26} H_{38} I_2.

Eüler and Nordenson [1] report the most recent investigations in regard to the carrot pigment. They found their crystalline preparation to be mixed with crystals of xanthophyll; they also showed that the belief often advanced that carotin is chemically related to cholesterol, is unfounded.

The Carotin of Green Plants.—Arnaud [2] was one of the first investigators to show that the carrot carotin is identical in properties with a yellow constituent of chlorophyll, although the existence of this yellow constituent of chlorophyll had long been the subject of investigation.

Berzelius [3] first sought to isolate a yellow pigment from autumn leaves by extracting with alcohol. He called it "Blattgelb" or xanthophyll, and expressed the belief that the pigment pre-existed along with the green coloring matter of the leaf.

The subject subsequently received the attention of many investigators. Fremy,[4] Michels, Millardet, Müller, Tinisnsseff, Gerland, Rannenhoff, Askennasy, Stokes, Sorby,[5] Tschirch,[6] Kraus,[7] Filhol,[8] Hansen,[9] Conrad,[10] Wiesner,[11] and many others took up the investigation.

Fremy designated the yellow pigment Phylloxanthin. Filhol noticed that by treating crude alcoholic chlorophyll solutions with animal charcoal it was possible to remove the green constituent of the mixture leaving a yellow colored solution, the color of which he believed was due to a pre-existing pigment or pigments associated with the green one. Kraus confirmed the observations of Filhol, and was the first to notice that when an alcoholic solution of chlorophyll is shaken with benzoline (petroleum ether) the alcohol retains the yellow coloring matter, the benzoline taking up the green constituent. Kraus' investigation was also the first to show that the ordinary chlorophyll spectrum was due partly to the green and partly to the yellow constituent, which he called xanthophyll. Kraus' xanthophyll gave a

1. Zeit. f. Physiol. Chem. 56, p. 223 (1908).
2. Compt. Rend. 100, p. 751 (1885); 104 p. 1293 (1887).
3. Ann. d. Chem. 21, p. 257 (1837).
4. Ann. Sc. Nat. 13, p. 45 (1860); Compt. Rend. 41, p. 189 (1865).
5. Proc. Roy. Soc. 21, p. 456 (1875).
6. Botan. Zeitung. 42, p. 817 (1884).
7. Flora, p. 155 (1875).
8. Compt. Rend. 39, pp. 9-184; 50, pp. 545 and 1182.
9. Sitz. ber. d. phys. Med. Ges. Würzberg (1883); and Arbeiten d. Botan. Gessel. Würzberg, 3, p. 127 (1884) and "Die Farbstoff des Chlorphylls" (1889).
10. Flora, Vol. 25 (1872).
11. Flora, Vol. (1874); Sitz. der. Wein. Akad. 89, 1. abts. p. 325.

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dark blue coloration with concentrated H_2SO_4, and bleached very quickly in the sunlight.

Sorby, using carbon bisulphide as the separator in place of benzoline, was the first to show that there is more than one yellow pigment associated with chlorophyll.

Hansen's method of isolating the yellow pigments was still different. He treated the alcoholic extracts with caustic alkali, evaporated the liquor to dryness and extracted the yellow pigment from the residue with ether, the spectroscopic study of which led him to believe that it exhibited three absorption bands. He believed also that it was identical with the pigment of the carrot.

E. Schunck [1] obtained from all crude alcoholic chlorophyll extracts minute sparkling red crystals which deposited on standing, and which he considered identical with the crystals which Bougarel [2] had called erythrophyll, and which Hartsen [3] has called crysophyll. This pigment showed two absorption bands.

Tschirch [4] using Hansen's method, found two yellow coloring matters, to which he gave the name xantho-carotin, showing three bands, and xanthophyll proper which showed no bands.

Returning now to Arnaud's [5] work, we find that he identified the red orange crystalline pigment which he obtained from spinach leaves with the carotin of the carrot, both as regards to crystalline form, melting point and chlorine derivatives.

We are indebted to Immendorff [6] for the confirmation of Arnaud's results indicating that the carotin of green plants is identical with the carotin of the carrot. Immendorff gave the pigment the formula which Arnaud found for carotin, namely $C_{26}H_{38}$. He states, however, that the percentage composition of the pure pigment corresponded best with Zeise's formula, C_5H_8. Immendorff believed that carotin was the only yellow pigment accompanying chlorophyll in the green leaf.

One of the most extensive publications in regard to carotin is that by F. G. Kohl [7] This author also gives one of the best and most voluminous compilations of the carotin literature that is to be found, besides a large amount of experimental data. The literature is also excellently reviewed by Tammes.[8] Kohl gave carotin the formula

1. Proc. Roy. Soc. 44, p. 449.
2. Ber. Chem. Gessel, 10, p. 1173 (1877).
3. Arch. Pharm. 207, p. 166 (1875).
4. Botan. Zeitung. 42, p. 817 (1884); Ber. der Deutsch. Botan. Ges. 14, pt. 2, p. 76 (1896).
5. Compt. Rend. 100, p. 751 (1885).
6. Landwirtschaftliche Jahrbücher 18, p. 507 (1889).
7. Untersuch, Uber d. Karotin, Leipzig. 1902.
8. Flora, p. 205 (1900).

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$C_{26}H_{38}$, and the iodine derivative $C_{26}H_{38}I_2$. He also gave a detailed description of the spectroscopic absorption of carotin. In ether and carbon-bisulphide he measured three bands:

	In ether		*In carbon bisulphide*
I	490-475 λ	I	510-485 λ
II	455-445 λ	II	470-458 λ
III	430-418 λ	III	437-425 λ

Carotin is lævorotatory, according to Kohl, α_D at 15° in chloroform being—30.17°.

Schunck [1] in his spectroscopic study of the yellow pigments of leaves and flowers, described the properties of carotin. Schunck also photographed the absorption bands of crysophyll (carotin) from the daffodil leaf, from spinach, from the carrot and from grass, in alcoholic solution. All of the carotin preparations showed the same three pronounced bands situated between F and H the first band of which lay almost directly upon the F line.

The most recent detailed investigation of the carotin of green plants is that of Willstätter [2] and Meig, and a study of their data shows that their results are to be accepted as the final proof of the chemical constitution and properties of this pigment.

Willstätter and Meig describe the properties of carotin as follows: Its crystals are copper colored plates of almost quadratic form, and melt at 167.5° to 168° C. Its crystals are soluble with great difficulty in hot ethyl alcohol and almost insoluble in cold ethyl alcohol, and in methyl alcohol they are still less soluble; one gram of the crystals requires 1.5 liters of petroleum ether (b. p. 30-50 °C.) for solution and about 900 c.cm. of hot ethyl ether; the crystals are difficultly soluble in acetone, easily soluble in benzol, very easily soluble in chloroform and instantly soluble in carbon bisulphide; the crystals are soluble in concentrated sulphuric acid with an indigo blue color and are precipitated as green flakes on dilution with water.

The carotin obtained by Willstätter and Meig crystallized from its deep red carbon bisulphide solution on addition of absolute alcohol, but analysis showed that the crystals contained ½ to ⅔ of a molecule of alcohol of crystallization. The carotin showed the composition of a pure hydrocarbon only after crystallization from low boiling point petroleum ether. From this solvent the preparation of Willstätter and Meig showed the composition C_5H_7. A preparation of carotin which the same authors obtained from the carrot showed the same

1. Proc. Roy. Soc. 72, p. 170 (1903).
2. Ann. der Chemie, 355, p. 1 (1907).

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composition. A molecular weight determination of both the carotin from the carrot and from the Brennessel leaves, by the ebullioscopic method in carbon bisulphide gave an average of 533 which corresponds exactly with $8(C_5H_7)$ or $C_{40}H_{56}$. This shows that Arnaud's formula of $C_{26}H_{38}$ is not quite correct. The same difference is brought out by the analysis of the iodine derivative which Willstätter and Meig also prepared.

The absorption bands of carotin were measured by Willstätter and Meig and they coincided almost exactly with those given for it by Tschirch [1] and Monteverde [2]. They did not attempt to measure the third band in the violet which they considered to be end absorption, but measured only the two bands in the blue and indigo blue.

Willstätter and Meig (alcohol sol.)	Tschirch (alcohol sol.)	Monteverde (petroleum ether sol.)
I 488-470 λ	I 487-470 λ	I 491-472 λ
II 456-438 λ	II 457-439 λ	II 461-444 λ

Carotin in Flowers, Fruits and Seeds. According to Czapek [3] carotins have been identified in many flowers by Hansen,[4] Immendorff,[5] Kohl,[6] Tammes,[7] Hilger,[8] and his pupils, Wirth,[9] Pabst,[10] Kirchner,[11] Ehrung[12] and Schuler.[13]

Among the fruits, Arnaud,[14] Passerini,[15] Kohl,[16] Schunck [17] and Montanari [18] have investigated the tomato pigment and believed it to be a carotin. Its identity as a truly isomeric carotin has recently been proved by Willstätter and Escher.[19] Schrötter [20] has shown that the pigment of the pumpkin is in all probability a carotin and Desmoliere [21] has identified carotin in the apricot.

1. Ber. d. deut. botan. Ges. 14, 76 (1896); 22, 414 (1904).
2. Acta Horti. Petropolitani XIII Nr. 9, 123 and 150 (1893).
3. Bichemie der Pflanzen, vol. I, p. 172, etc.
4, 5, 6, 7. Loc. cit.
8. Botan. Centr. 57, p. 335 (1894).
9. Dissert. Erlangen (1891).
10. Arch. Pharm. 230, p. 108 (1892).
11. Dissert. Erlangen 1892.
12. Botan. Cent. 69, p. 154 (1897).
13. Dissert. Erlangen. 1899.
14. Compt. Rend. 102, p. 1119 (1886).
15. Compt. Rend. 100, p. 875 (1885).
16. Loc. Cit.
17. Proc. Roy. Soc. 72, p. 172 (1903).
18. Le Staz. sp. agra. ital. 37, p. 909 (1904).
19. Zeit. Physiol. Chem. 64, p 74 (1910).
20. Vehr. Zool. bot. Gessel. 44, 298 (1895).
21. Chem. Centr. 2, p. 1001, 1902.

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Among the seeds, Schunck [1] has found the annatto pigment to be a carotin.

The Xanthophylls.

It was mentioned above that it has been found that a second class of pigments usually accompanies carotin. Investigations of this class of pigments, now called xanthophylls, has not been as extended as that of carotin but the constitution and properties of the xanthophylls are nevertheless at present established.

Sorby [2] differentiated the pigments accompanying chlorophyll as xanthophyll, orange xanthophyll, and yellow xanthophyll, all with spectroscopic properties. J. Borodin [3] observed that besides carotin, a second crystallizable yellow substance exists in leaves which is much more soluble in alcohol than carotin and insoluble in benzine. Immendorff [4] denied the existence of more than one pigment as was noted above. Monteverde [5] confirmed Borodin's observations. Tschirch [6] in 1896, showed that green leaves contain a second yellow pigment which, however, showed no absorption bands. Tschirch called the second pigment xanthophyll. The name, however, was a misnomer, for Schunck [7] later showed that Tschirch was dealing with a group of water and alcohol soluble pigments probably identical with the lichnoxanthine described by Sorby.[8] Tschirch [9] later recognized the existence of a true second yellow crystallizable pigment.

Molisch [10] in his critical study of the yellow pigments left the question of their plurality an open one, and Tammes [11] also left the question undecided.

Schunck [12] in his widely known spectroscopic study of the yellow pigments of plants and flowers, demonstrated beyond a doubt that a second great group of pigments, which he designates the xanthophylls, accompanies the crysophyll. He differentiated three different xanthophylls and designated them L. B. and Y. xanthophyll, respectively.

He found that the xanthophylls were all characterized by giving the same color reactions in the dry state as crysophyll and three

1. Proc. Roy. Soc. 72, 1903.
2. Proc. Roy. Soc. 21, p. 457 (1875).
3. Melanges Biol. tir. d. bull d. L'Acad. Imp. d. St. Petersb. 11, p. 512 (1883).
4. Loc. cit.
5. Loc. cit. p. 148 (1903).
6. Ber. d. d. Botan. Gessel, 14, p. 76 (1896).
7. Proc. Roy. Soc. 72 (1903).
8. Loc. cit.
9. Ber. d. d. Botan. Gessel. 22, p. 414 (1904).
10. "Die Krystallization und der Nachweis des Xanthophylls (carotins) in Blatte" (Ber. d. Deut. Botan. Ges. 14, p. 18 (1896).
11. Loc. cit. (1900).
12. Proc. Roy. Soc. 65 (1899); 68 (1901); 72 (1903.)

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similar absorption bands in the violet region of the spectrum. The bands of the xanthophylls, however, were all shifted somewhat towards the blue with respect to the bands of crysophyll, the amount of shifting depending on the xanthophyll, L xanthophyll being shifted the least and Y xanthophyll the most. Schunck found the absorption bands of the different xanthophylls especially characterized by the action of their alcoholic solutions in the presence of HCl and HNO_3 the details of which are given in his latest paper.[1]

Schunck also made the very interesting discovery that the yellow pigment of egg yolk and fowl serum shows the identical properties of L xanthophyll both with respect to the position of the original absorption spectra and also the action of acids upon the spectra.

One of the most interesting and important studies of chlorophyll and its accompanying yellow pigments was made by Tswett[2] who discovered and thoroughly investigated the adsorption properties of these pigments. He was able to demonstrate the presence of at least four different xanthophylls which he designates as xanthophylls α α' α'' and B. A more detailed review of this work will be given in connection with a report of the present investigations. It is of interest here especially on account of its historical position with respect to the establishment of the chemical constitution of the xanthophylls.

It was Willstätter and Meig[3] who isolated and identified the crystalline xanthophyll pigment accompanying the carotin in green plants and leaves, and, as noted above, Eüler and Nordenson[4] have recently found xanthophyll crystals in their extracts from the carrot, thus indicating a more general distribution of the xanthophylls in connection with carotin than has been believed.

The results of the study of the crystalline xanthophyll show that it is composed of carbon, hydrogen and oxygen in the proportion $C_{40}H_{56}O_2$ and is thus merely carotin dioxide.[5] The pigment is further distinguished from carotin by the color and shape of its crystals, which are yellow or orange trapesium plates sometimes spear or wedge-shaped which are characterized by a steel blue reflection. The pigment exhibits an entirely different solubility toward petroleum ether and absolute alcohol than carotin, being insoluble in the former and readily soluble in the latter solvent. According to these authors, the pure

1. Proc. Roy. Soc. 72 (1903).
2. Ber. Botan. Gessel, 24, pp. 316 and 384 (1906).
3. Ann. der. Chemie, 355, p. 1 (1907).
4. Loc. cit.
5. Willstätter and Meig point out the probable identity of xanthophyll with the hitherto unexplained hydrocarotin found by Husemann.

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crystals have a melting point of 172° C. (corrected) which is slightly higher than the melting point of the carotin crystals; and the absorption bands of the pigment are slightly shifted toward the violet from the corresponding bands of carotin, as was also shown by Schunck [1] for the xanthophylls which he differentiated.

It might be readily assumed that xanthophyll is formed directly from carotin in the plant. In fact Tschirch [2] has claimed that carrot carotin goes over to xanthophyll in the air. Eüler and Nordenson [3] do not credit this statement and state that, "One may well suppose that in the plant, xanthophyll normally is formed from the carotin, but outside of the plant it has not been possible to imitate this transformation, the most skillful oxidation always leading to a much higher oxidized product." Willstätter and Meig believed in this connection that xanthophyll although carotin dioxide is not the end product of the oxygen absorption of carotin in the plant. Monteverde [4] and Lyubimenko have recently claimed that chlorophyll and xanthophyll originate from the same colorless substance, carotin being a complimentary product generated during the formation of cholorophyll, but not necessarily from the xanthophyll.

The Pigments of Animal Origin.

The Luteins.—We will now direct our attention to a review of the literature bearing upon the yellow pigments of so-called animal origin. Thudichum [5] was one of the first to investigate the yellow animal pigments and he classified a great many of them together with the yellow pigments of plants under the name lutein, the name being taken from the pigment of the corpus luteum. He states, "Various parts of animals and plants contain a yellow crystallizable substance which has hitherto not been defined, and which I call lutein. It occurs in the corpora lutea of the ovaries of animals, the serum of the blood, the cells of adipose tissue, in butter, in the yolks of eggs of oviparous animals, in seeds such as maize, in husks and pulps of fruits such as annatto, in roots such as carrots, in leaves such as those of coleus, and in the stamens and petals of a great many flowers."

It is unfortunate that none of the above statements are supported by experimental evidence, for it can hardly be accepted that Thudichum was able to obtain crystals of lutein from all the bodies in which he

1. Loc. cit.
2. Ber. Botan. Gessel. 22, p. 414 (1904).
3. Loc. cit.
4. Bull. Acad. Imper. Sc. St. Petersb. 30, p. 609 (1912).
5. Proc. Roy. Soc. 17, p. 253 (1869).

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claims to have found it, or was able to show all the properties which he describes for the crystals which he evidently did obtain.

Crystalline animal pigments were apparently obtained before Thudichum's claims in this regard. According to Krukenberg,[1] Wittich[2] obtained crystals of a red pigment from Euglenia Sanguirubo, and Piccolo and Lieben[3] found a crystalline animal pigment. Pouchet[4] a little later obtained a yellow crystalline pigment from lobsters.

The Chromophanes.—The early workers in the field of animal pigments laid great emphasis upon the so-called color reactions, one of which, the blue reaction which concentrated HNO_3, was mentioned by Thudichum. That a similar reaction is given by concentrated H_2SO_4 was first noticed by Wittich in 1863, and Buchholz also noticed it with a fat pigment from a Ganglion cell of an invertebrate. Piccolo and Lieben had also noticed the blue reaction with concentrated H_2SO_4. Besides Thudichum, Filhol[5] and Städeler[6] noticed the blue reaction with concentrated HNO_3. Städeler attempted to isolate the egg yolk pigment. He failed to do so, however, but attempted to establish the difference between it and Bilirubin with which it had been considered identical. A little later a third reaction of the luteins was discovered by Schwalbe,[7] namely a blue-green color with a solution of iodine in potassium iodide. Schwalbe first noticed the reaction with the cone-globules of the retinas of birds and lizard's eyes. The red globules gave a beautiful blue to blue-black color, and the yellow oil globules a green to blue-green to blue. The pigments thus characterized were called chromophanes by Schwalbe and the existence of these pigments was a little later considerably extended by Capranica[8] who also made use of the iodine reaction.

Kühne[9] took up the study of the chromophanes of the cone-globules of bird retinas, and separated three pigments which he designated Rhodophan, Chlorophan and Xanthophan, respectively, according to the color of their solutions.

Kühne also studied the absorption spectra and color reactions of the pigment of the egg yolk and the corpus luteum and compared them

1. Grundzuge einer vergleichenden Physiologie der Farbstoff und der Farben; 1884.
2. Arch f. Path. Anat. 27, p. 573 (1863).
3. Giornals d. Scienze Naturali et. Economich. Palermo 2, p. 258 (1866).
4. Jour. d. L'Anat. et. Physiol. 12, p. 12 (1876).
5. Compt. Rend. T. 39, p. 184, T. 50, pp. 545 and 1182.
6. Jour. f. Pract. Chem. 100, p. 149 (1867).
7. Hand D. Ges. Augenheilkunde von Graefe u. Saemisch I, p. 414 (1874).
8. Arch. f. Anat. Physiol. p. 283 (1877).
9. Untersuch, des Physiol. Universität Heidelberg I, 4th Heft, p. 341 (1878); IV, p. 169 (1882); Jour. Physiol. 1, p. 109 (1878).

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with these properties of the retinal pigments. A study of their spectroscopic absorption properties led him to believe that the pigments were not identical.

Kühne in his celebrated work on "Optochemie" occupied himself somewhat again with the egg yolk pigment and called it Ontochrin or Lecithochrin. He did not try to isolate it free from nitrogen, but he did succeed in observing crystals. He again was careful to distinguish between the egg yolk pigment and the corpus luteum pigment, which he at this time considered as extraordinarily closely related to carotin.

The Lipochromes. Basing his work on the researches of Kühne, Krukenberg commenced a series of researches which extended from 1879 to 1886, the most important of which appeared in his "Vergleichende Physiologische Studien" [1] and especially in the paper, "Grundzuge einer vergleichenden Physiologie der Farbstoff und der Farben" which appeared in 1884. Krukenberg made an exhaustive study of what had been done on animal pigmentation and included under one head all those pigments which had previously been known as luteins, carotin, zoonerythrin (tetronerythrin) and Kühne's chromophanes, and called them lipochromes.

Krukenberg believed that carotin, the pigment of the carrot, was the best representative of the lipochrome coloring matters, and accepted Husemann's formula for carotin ($C_{18}H_{24}O$) as representing the chemical composition of the lipochromes.

In regard to the origin of lipochromes Krukenberg believed, "It is probable that in most cases they originate from fatty substances, for frequently, if not without exception, they occur in company with fat and allow themselves to easily go over into cholesterin-like bodies."

In 1885 Krukenberg [2] isolated a yellow lipochrome from the blood serum of the ox by extracting the serum with amyl alcohol. The solution showed two absorption bands, one enclosing the line F and the other lying between F and G. A year later Halliburton [3] reported that he extracted a yellow lipochrome from the blood serum of the pigeon, hen, dove and tortoise by means of alcohol. Halliburton reported an identical pigment in the body fat of these same animals.

MacMunn [4] was the next investigator of animal pigments, and like Krukenberg, he extended the classification lipochrome to include

1. Zoonerythrin (Tetronerythin):—Central. f. d. Medic. Wiss. 1879. Vergl. Physiol. Studien I Reihe, II Abth. s. 67-71; III Abth. s. 114-115; IV Abth. s. 30-35; V Abth. s. 87-94; II Reihe, I Abth. s. 165-167; III Abth. s. 135).
2. Sitz, ber. d. Jen. Gessel. f. Med. 1885.
3. Jour. Physiol. 7, p. 324 (1886).
4. Philos Trans. Roy. Soc. 177, p. 247 (1886).

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the yellow constituent of chlorophyll or Hansen's "Chlorophyll Yellow." He believed that the lipochromes were chemically closely related to chlorophyll.[1]

MacMunn's greatest contribution to animal chromotology was in 1889.[2] The pigments of a great many marine animals, Crustacea, worms and sponges were examined and classified. Lipochromes were found abundantly, MacMunn drawing a distinction as to whether the lipochrome was a rhodophan or a chlorophan-like lipochrome.

In regard to some of the properties of the lipochromes MacMunn states, as did Krukenberg, that they are sensitive to light, both in the solid state and in solution, and yield in many cases cholesterin-like substances. He believed that many of the plant lipochromes were identical with the animal lipochromes.

It will be remembered that for a long time there were many followers of the view that a close relationship existed between carotin and cholesterol and that this view was only finally discredited by a study of the pure crystalline pigment.

Cotte[3] recently carried out an investigation in which he sought and claims to have shown that the lipochromes, both animal and vegetable are intimately associated with cholesterol. Cotte's results have been thoroughly disproved by Henze.[4]

Since the early work of Pouchet[5] and Maly[6] who distinguished between yellow and red crustacean lipochromes many investigators have classified the lipochromes according to their red or yellow color. Newbigin[7] in a recent investigation of the pigments of the skin, muscle and ovaries of the salmon, reports that he found two pigments present, a red and a yellow, which he claims he was able to separate from each other. Newbigin concluded from the color reactions of the pigments that the red pigment was a true lipochrome while the yellow pigment was not.

In regard to the yellow pigment, Newbigin says that, "It belongs to a group of pigments that are apparently exceedingly widely distributed in the animal kingdom, but which have been little investigated. They have been commonly confounded with the lipochrome pigments."

He extracted the pigment from the bright yellow body fat of a cow and found it to have properties identical with the yellow pigment

1. Jour. Physiol. 9, p. 1 (1888).
2. Quart. Jour. Micros. Sc. 30, p. 15 (1889).
3. Compt. Rend. Soc. Biol. 55, p. 812 (1903).
4. Zeit. Physiol. Chem. 41, p. 109 (1904).
5. Jour. d. l'Anat. de la Physiol. 1, 12, 10 (1876).
6. Sitz. d. k. Akad. d. Wiss. zu. Wein. 83 (1831).
7. D. Nöel Patton—Report of Inv. on Life Hist. of Salmon (1898), Article XV.

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of the salmon with the exception that it was very little soluble in methyl alcohol, but dissolved readily in ether.

General Properties of the Lipochromes. It will not be out of place to give a brief summary here of the general characteristics and properties of the lipochrome pigments as found up to this time.

Lipochromes[1] may be classed as salve-like, yellow or red or orange colored residues, which have been obtained in needles or rhombic plates, where they have been crystallized. They are soluble in alcohol, ether, benzol, petroleum ether, amyl alcohol, chloroform, carbon bisulphide, ethereal oils and fats with a yellow or yellow-orange color. They are insoluble in cold and hot water and alkalies and dilute acids, but are soluble in alcoholic alkaline solutions and are unchanged when these solvents are heated. In alcohol or other solvents they are unstable, and readily bleach, as do the residues from these solutions. The bleach product is unknown, but it is certainly not identical with cholesterol. On addition of concentrated H_2SO_4 or HNO_3, the lipochromes give a color change of blue-green-violet to brown. The color reactions are often interfered with by the presence of a small amount of foreign substance. The lipochromes generally give a blue-green coloration with a solution of iodine in potassium iodide. Spectroscopically the lipochrome solutions show two bands and sometimes three in the blue part of the spectrum, and again they sometimes show no bands at all.

The lipochromes may be extracted from the fresh or dried tissues in which they are found, by organic solvents, best by hot or cold alcohol, ether, petroleum ether, carbon bisulphide or chloroform, the choice of the solvent resting with whether some foreign pigment is present. When fat is present, the pigment may be heated with alcoholic alkali which will not saponify the lipochromes. The lipochromes can be extracted from the soap with ether, petroleum ether, or chloroform, either directly or after acidifying, or the lipochromes can be salted out of their alkaline soap solutions with sodium chloride, and the lipochromes obtained by extracting the precipitated soap with alcohol or ether.

The Lipochromes of Algae, Fungi, and Bacteria. While a wide distribution of the lipochromes has already been mentioned, a review of their literature would not be complete without mentioning their distribution in algae, fungi and bacteria.

1. Summarized from "Lipochromes" by Franz Samuely. Alderhalden's Biochemisches Handlexikon vol. 6, and Handbuch der Biochemischen Arbeitsmethoden, vol. 2.

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Hansen[1] first showed the presence of lipochromes in algae and Tammes[2] has lately shown their presence in a large number of these plants. Zopf[3] has investigated the lipochromes of fungi and especially of bacteria, the first lipochrome-producing bacteria being pointed out by him.

The Lipochrome or Lutein of Egg Yolk. It will be readily agreed that while some order has been attained in classifying the widely distributed animal pigments, by means of the convenient and flexible classification "lipochromes," our knowledge of the animal pigments is far from being satisfactory when compared with the status of the orange and yellow plant pigments, the carotins and xanthophylls. The science of animal chromotology should accordingly be exceedingly grateful for the recent work of Willstätter[4] and Escher, on the lutein of egg yolk, the result of which has been to throw new light upon the constitution of the lipochromes of the higher animals and upon their relations to the carotins and the xanthophylls.

The main pigment of the yolk of hen's eggs was isolated in crystalline form by these investigators, and when in approximately pure condition showed sufficiently close agreement with the constitution of xanthophyll that the authors claim that the egg lutein on account of its melting point (195-196° C. corrected) is a true isomer of the crystalline xanthophyll of green plants. In all its other properties including its spectroscopic absorption bands, the egg lutein was identical with the crystalline plant xanthophyll.

It is worthy of note also that during the isolation of lutein a minor constituent was noticed which gave every indication of being closely related to carotin; but as it was present in very small amount compared with the xanthophyll it was disregarded.

In concluding the review of this investigation it will be important to mention that the authors state that one of them, i. e., Escher, is at present investigating the pigment of the corpus luteum which they state has been found to belong to the hydrocarbon or carotin group of pigments.[5]

1. Arbeit. Botan. Inst. Würzberg 3, 296 (1883).
2. Loc. cit.
3. Ber. Botan. Gessel. 9, 27 (1891).
4. Zeit. Physiol. Chem. 76, pp. 214-225 (1912).
5. Note—Since writing the above, Dr. Escher has published his investigations which show that the corpus luteum pigment is in every respect identical with the carotin of the carrot and of green plants. Zeit. f. Physiol. Chem. 83, p. 198 (1913).

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The Physiological Relation Between Plant and Animal Lipochromes.

With the review of the chemical side of this problem complete, it yet remains to consider what has been shown in regard to relations other than chemical, between the animal and plant pigments whose properties are so nearly related and in many cases identical.

The literature has been found to be very brief on this point. Newbigin [1] gives a rather extensive consideration of this subject and attempts to explain the presence of the red and yellow pigments found by him in the salmon organism. While he considered the most obvious explanation to be that they were derived directly from the food, he found a number of difficulties in the way of the acceptance of such an explanation, the most important of which was that he was able to show the presence of but a trace of only the yellow pigment in the usual food of the salmon.

As to the possibility of transferrence of yellow pigments from one organism to another, Newbigin points out what he believes to be some evidence apart from the case of the salmon. He says, "Poulton [2] has shown by experiment that certain caterpillars derive their pigments from their food. Again it is not uncommon to find fat of sheep and cows dyed a deep yellow color. According to some authorities this occurs quite sporadically without known cause, while according to others, special foods, notably maize, are the important agents." Newbigin says in this connection, "I have examined the yellow pigment of maize, and compared it with the pigment from yellow fat. The maize pigment gives the lipochrome reaction faintly with H_2SO_4 distinctly with HNO_3 while the fat pigment gives no lipochrome reaction. In other respects, in tint, solubility, etc., the pigments closely resemble each other." Newbigin did not feel warranted to conclude from these experiments that all yellow pigments of animals are derived from their food, for with such a conclusion, he states, "It would be difficult to understand why such colored fat should not be universal in herbivorous animals, for all green parts of plants contain also a certain amount of yellow pigment."

It seemed to Newbigin, however, that a reasonable explanation for salmon, domesticated cattle and caterpillars would be to suppose that when they ingest a moderate amount of colored fat in their food, that they could utilize or eliminate the pigment, and so deposit colorless fat in the tissues; but when the ingestion of colored fat is in excess of the actual requirements as it so often is, especially with domes-

1. Loc. cit.
2. Proc. Roy. Soc. 54, p. 417; Nat. Sci. 8, p. 98.

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ticated cattle, an elimination or utilization of the pigmented fat is impossible and fat colored with the pigment in a more or less modified condition is thus stored up.

There is abundant proof in this literature aside from the above speculations that animals are able to lay up fat soluble dyes in the organism and even eliminate them in the milk. Only recently Mendel and Daniels [1] have shown that Sudan III and other fat soluble dyes may be deposited in the organism in adipose tissue and bone marrow when introduced into the organism either dissolved in fat or when fed alone. When fed with fat or when fat was present in the alimentary tract the dyes entered the organism through the lymphatics in solution in fat, but when fat was absent, through the portal circulation dissolved in bile in which they are nearly all soluble. In the latter case the pigments did not pass beyond the liver unless fat was present to transport them, in which case only they were subsequently found in the blood. When fat stained food was fed to small animals (cats, rats, guinea pigs, etc.) in lactation, and in one case with a goat, the dye appeared in the milk shortly after the first feeding of the dye. The same authors feeding fifteen grams of Sudan III to a Holstein cow for three successive days were unable to detect the dye in the milk. The authors also made the interesting observation that stained fat does not traverse the placental barrier; the blood and foetus and fat of the young born of Sudan-stained female cats and rats were free from the dye.

Gogitidse [2] fed hog fat (100 grams per day) colored with Sudan III to a bitch and after two days found the dye in the milk. The body fat did not show this coloration so soon and then not so clearly, in fact only after long continued feeding of the stained fat.

Backhaus [3] studying the "Influence of Feed and Individuality on the Taste and Healthfulness of Milk," says that a number of plants influence the color of milk and butter. The same author conducted several pigment feeding experiments with cows. Negative results were obtained with respect to the milk when feeding Fuchsin, Bismark brown, and curcuma powder, although the feces showed the pigments abundantly. When feeding sodium fluorescin the urine was affected but not the milk. When feeding methyl violet, however, the author was able to show that this pigment was carried into the milk fat in a reduced condition so that on contact with the air and with the aid

1. Jour. Biol. Chem. 13, No. 1, p. 72 (1912).
2. Zeit. f. Biol. 45, 353 (1904).
3. Berichte, Landwirt. Inst. U. Königsberg 5 (1900).

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of heat, the milk fat showed an intense blue coloration. The feces, however, showed the pigment in an unchanged condition.

Summary.

The foregoing review of the literature has shown that the great number of pigments that exist throughout the entire plant and animal kingdoms have long been of interest from a scientific standpoint. The pigments of botanical origin have been thoroughly and exhaustively investigated. This is especially true of the yellow pigments carotin and xanthophylls, and their chemical constitution and properties are now established.

The yellow and orange pigments of plants were at first classified in one group, and were called carotins, the name being derived from the pigment of the carrot, which was the first one investigated. A great many different names were given to this pigment as it was independently discovered in various plants but the identity of these pigments with the carrot pigment has now been established. It was eventually discovered that the carotins are always accompanied, especially in green plants, by a second great class of pigments which have been called xanthophylls, whose relation to carotin has but recently been established.

As the work on plant pigmentation developed, it was recognized that the general properties of a great many yellow pigments found in animals were similar to the so-called carotins. The first investigators classified these animal pigments under the name lutein, the name being derived from the pigment of the corpus luteum, which was the first one investigated. The name lutein was extended by the animal chromotologists to include the carotins of plants and its related pigments. Later, when the animal luteins had become generally recognized by their association with fat, the name lutein was changed to lipochrome and this designation was also extended to include all similar pigments of both plants and animals.

The classification of the plant and animal pigments which is at present generally accepted is to restrict the names carotin and xanthophylls to the two great classes of yellow plant pigments, and to include under the name lutein or lipochrome only those yellow pigments which are considered to be of animal origin.

The most recent work in the field of animal chromotology has shown that the luteins can also be subdivided into carotin and xanthophyll groups depending on their chemical relation to the carotin or xanthophylls of plant origin. Accordingly Schunck [1] has shown the

1. Loc. cit.

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spectroscopic identity of the egg yolk pigment with a xanthophyll which he isolated from the yellow daffodil, the nasturtium, and green leaves. Willstätter and Escher [1] have confirmed this with a chemical analysis of the egg yolk pigment, showing it to be a true isomer of the crystalline xanthophyll of green plants; they have called it xanthophyll B. Escher [2] has recently published his investigation showing that the pigment of the corpus luteum is identical in chemical composition and properties with the carotin of green plants.

These recent discoveries have opened the way for an extension of such investigations to other yellow animal pigments whose isolation is rendered much more difficult by their association with very large quantities of fat and other substances. These discoveries have also raised the question whether any relation other than chemical exists between the yellow animal and plant pigments. This question has never been investigated. The investigations which will be reported in the succeeding papers are the first to show that there is a definite relation other than chemical between the yellow plant and animal pigments.

1. Loc. cit.
2. Loc. cit.

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RESEARCH BULLETIN NO. 10

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AGRICULTURAL EXPERIMENT STATION

CAROTIN—THE PRINCIPAL NATURAL YELLOW PIGMENT OF MILK FAT—PART II

Chemical and Physiological Relations of Pigments of Milk Fat to the Carotin and Xanthophylls of Green Plants

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COLUMBIA, MISSOURI
April, 1914

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CAROTIN—THE PRINCIPAL NATURAL YELLOW PIGMENT OF MILK FAT—PART II.*

Chemical and Physiological Relations of Pigments of Milk Fat to the Carotin and Xanthophylls of Green Plants.

LEROY S. PALMER AND C. H. ECKLES

For a number of years the great variety of yellow animal pigments have been classified under the general name lipochrome. Recent investigations by Willstätter and Escher,[1] and by Escher[2] have shown, however, that some of these pigments are in reality very closely related or identical with the carotin or xanthophyll pigments of plants. Willstätter and Escher have analyzed the pure lutein of egg yolk and found it to be isomeric with the crystalline xanthophyll of green plants; and Escher has identified in the same manner the lipochrome of the corpus luteum as a true carotin. The isolation of both pigments was attended with great difficulty. In the case of the egg yolk pigment the yolk of 6,000 hen eggs yielded only four grams of crude crystalline pigment, while in the case of the corpus luteum pigment less than 0.5 gram of crystals were obtained from 10,000 cows' ovaries.

The natural yellow pigment of butter is the most commonly observed of all animal lipochromes. It is also more important from a commercial standpoint than any other animal pigment; the public judges the richness of dairy products by their yellow color, and demands that butter especially shall have a standard shade of yellow. The pigment of butter fat, however, has been the least investigated of all animal lipochromes. Thudichum's[3] classical investigation included the pigment of butter fat under the general classification lutein, which he proposed. No other study of the butter fat pigment has

1. Zeit. f. Physiol. Chem. 76, pp. 214-225 (1912).
2. Zeit. f. Physiol. Chem. 83, p. 198 (1913).
3. Proc. Roy. Soc. 17, p, 253 (1869).

* See Res. Bul. No. 9, p. 312, for statement of co-operation with U. S. Dept. of Agriculture.

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been reported. It is usually classified in the current text books,[1] however, according to Krukenberg's classification of lipochromes.

In view of the commercial importance attached to the butter fat pigment and especially in view of the results of the most recent investigations in this field, it was recognized that a thorough investigation of this pigment would be of great value both from a scientific as well as a practical standpoint.

The present investigation was therefore undertaken for the purpose of classifying the butter fat pigment as a true lipochrome and also with respect to its relation to the carotin and xanthophylls of green plants. It was also the purpose of the investigation here recorded, to gather as much information as possible relative to the influence of certain factors upon the color of butter, among which may be mentioned the character of the ration and the breed of the cow.

METHODS OF ISOLATION.

The statement is frequently met in the literature[2] and in the text books and works[3] on oils and fats, that the pigment of butter or butter fat appears in the unsaponifiable extracts along with cholesterol and other substances.

A number of methods for obtaining the unsaponifiable matter of butter fat are available and several were tried. The method finally adopted for the isolation of the crude pigment was to saponify the butter fat with a twenty per cent solution of alcoholic potash, using 2 c. c. for each gram of fat. Saponification was allowed to continue for one half to one hour at the temperature of the boiling solution. The soap was dissolved in three volumes of distilled water. After cooling, the solution was shaken with an equal volume of pure ether in a separatory funnel. The extraction was repeated with a fresh volume of ether equal to one-half the volume of the soap solution. It was found that this procedure would leave the soap colorless, if no aldehyde resins had formed during saponification or none of these colored bodies had been present in the alcoholic potash previous to its addition to the fat. The ether extract containing the pigment and other unsaponifiable matter was now freed from alkaline soap, by shaking many times with excess water, carefully at first to avoid

1. Such as Hammarsten, "Text Book of Physiological Chemistry" and Schaefer, "Text Book of Physiological Chemistry", etc.
2. Kirsten: Zeit. Nahr. Genuss. 5, p. 833 (1903).
3. Lewkowitsch: "Oils, Fats and Waxes." Vol. I, p. 371, (1909 Edition).

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emulsions, and more vigorously with subsequent washings. When the wash water no longer reacted alkaline toward phenolphthalein, the ether was either dried over fused $CaCl_2$ or, after standing several hours, decanted from the precipitated moisture. The ether was then evaporated, leaving a salve-like residue of various tints of yellow to orange to red, depending upon the amount of fat used and the depth of its original color.

Although the methods of study eventually adopted did not make the procedure necessary, it was found possible to completely free the pigment from its chief impurity, i. e., cholesterol, by means of the digitonin method of Windaus [1] for the quantitative estimation of cholesterol. A hot one per cent solution of digitonin in ninety per cent alcohol, when added to an alcoholic solution of the unsaponifiable residues from butter fat, completely precipitated the cholesterol as a colorless compound, leaving the pigment in solution. This solution was still contaminated, however, with traces of fat and lecithin decomposition products.

GENERAL PROPERTIES OF THE BUTTER FAT PIGMENT.

The unsaponifiable residues from butter fat, either crude or freed from cholesterol by digitonin, were readily soluble in hot alcohol and in ether, chloroform, petroleum ether, etc. with a golden yellow color; and in carbon bisulphide with a color which varied, according to the concentration, from a red orange to a blood red color. The freshly prepared crude, and also, the cholesterol-free residues usually gave an instantaneous but transient purple color on adding a drop of concentrated sulphuric acid, a light green color quickly changing to a greenish blue color on adding a drop of concentrated nitric acid, and a dark blue color with the combined acids. These color reactions were usually shown a little clearer by the cholesterol free residues. Small amounts of impurities often interfered with the color reactions; in fact they were often rendered negative by some slight decomposition of the pigment which was not apparent in the intensity of the color. This was due to the fact that the crude samples of butter fat pigment were very unstable, quickly bleaching in the air, especially with the aid of heat in the presence of a little water. It was necessary therefore to take great care to have the ether solutions of the pigment as free as possible from water before evaporation, or to transfer the pigment to some solvent such as petroleum ether, which does not absorb water so readily.

1. Zeit. f. Physiol. Chem. 65, p. 110 (1909).

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In addition to the above properties it was found that all fat-free, but not necessarily cholesterol-free solutions of the pigment showed spectroscopic absorption bands. In alcohol two sharp bands were exhibited in the blue part of the spectrum; and in carbon bisulphide these bands were nearly always accompanied by a third faint band in the violet, which was now visible on account of the general shifting of the bands toward the red end of the spectrum. The unstable character of the butterfat pigment required that the isolation be carried out as rapidly as possible in order to preserve all the characteristic properties of the pigment. This was especially true for the study of the absorption spectra. Fifteen to thirty grams of fat were found to yield sufficient pigment for a spectroscopic study. The use of large quantities of fat (300 to 1,000 grams) always led to unsatisfactory results.

The general properties of the butter fat pigment show that it is to be classed as a true lipochrome. The chemical relation of the pigment to the carotin and xanthophylls of green plants remains to be shown.

METHODS OF IDENTIFICATION.

The nature of the substances with which the butter fat pigment is associated at once precluded its isolation in sufficient quantity to establish its chemical composition and molecular weight. It was therefore necessary to adopt other methods of identification which would be sufficiently accurate and characteristic that the final results could not be mistaken.

The methods that were adopted were, (1) a study of the spectroscopic absorption properties, (2) a study of the relative solubility properties, (3) a study of the adsorption properties with respect to calcium carbonate; (4) an attempt was also made to study the crystalline form.

Before giving the results of our studies, some discussion will be given of the relative solubility and adsorption properties of carotin and the xanthophylls.

RELATIVE SOLUBILITY OF CAROTIN AND XANTHOPHYLLS.

M. Tswett[1] was the first one to publish a comprehensive statement in regard to the relative solubility of the plant pigments in

1. Ber. der. Deut. Botan. Gessel. 24, pp. 316, 384 (1906).

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different solvents. He classified the solvents into three groups as follows according to their relation to the leaf pigments.

"1. Alcohols (methyl, ethyl, amyl), acetone, acetaldehyde, ether, chloroform: These solvents, acting on freshly cut up or dried leaves dissolve out all pigments equally and completely."

"2. Petroleum ether or petroleum benzin: This solvent, acting on fresh leaves finely ground with sand or emery, takes on a more less yellow appearance, which is especially due to carotin, but contains also other pigments. Dried leaves (at a low temperature) likewise give up their carotin to this solvent, and in somewhat purer condition."

"3. Benzol, xylol, toluol, and carbon bisulphide: These solvents set intermediately between the first two groups."

Willstätter and Mieg [1] a little later approached the same problem from another standpoint and showed that the methods used by Kraus [2] and Sorby [3] for demonstrating the presence of more than one pigment in green plants, when properly applied could be made characteristic properties of carotin and xanthophylls. Kraus shook his alcoholic extracts of green leaves with petroleum ether and found that the green pigment went into the petroleum ether leaving the alcoholic solution yellow. Sorby shook his alcoholic extracts with carbon bisulphide and found that the latter solvent contained the green pigment while the alcohol was left yellow. Wilstätter and Mieg, applying these tests to the isolated carotin and xanthophyll pigments obtained the following results:

"1. If methyl alcohol is added to a petroleum ether solution of carotin so that the liquids do not mix, the carotin will remain for the greatest part in the petroleum ether layer, the alcohol layer being only slightly colored. If a trace of water is added, the methyl alcohol layer will become colorless. The same phenomenon occurs with ethyl alcohol, and one can start with an alcohol or benzol solution and show the same thing."

"2. If carbon bisulphide is added to an alcoholic carotin solution and a little water added, the carbon bisulphide will separate and will quantitatively contain the carotin."

"3. If an alcoholic solution of the xanthophylls is mixed with petroleum ether and the liquids separated with a little water, by far the greatest portion of the xanthophylls will be found in the alcohol layer."

1. Ann. der. Chemie 355 p. 8 (1907).
2. Flora, p. 155 (1875).
3. Proc. Roy. Soc. 21, p. 456 (1875).

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"4. If an alcoholic solution of xanthophylls is mixed with carbon bisulphide and the solvents separated with water, the xanthophylls will be distributed between both layers."

Schunck,[1] using Sorby's method, showed that if alcoholic solutions of xanthophylls are repeatedly shaken with carbon bisulphide all the xanthophylls with spectroscopic absorption properties can be extracted.

Tswett[2] studying this question again in 1911 states that, "According to a well known rule, organic compounds are best soluble in solvents of similar composition," and concludes that carotin, a hydrocarbon, is therefore much more readily soluble in the hydrocarbons of the aliphatic and cyclic group than in alcohols, a point well illustrated by the above experiments of Willstätter and Mieg. Continuing, Tswett states, "If one therefore shakes an eighty to ninety per cent alcoholic solution of carotin with petroleum ether, the pigment goes almost completely into the petroleum ether layer." "A pigment which in the above mentioned two phase system occupies the lower alcoholic layer, is therefore not a carotin." It may be added in the light of Willstätter and Mieg's investigation that if the original solution before differentiation was a mixture of carotin and xanthophylls, the lower alcoholic layer will contain the xanthophylls.

ADSORPTION PROPERTIES OF CAROTIN AND XANTHOPHYLLS.

Considering now the so-called adsorption properties of the pigments, we find that this striking characteristic was discovered and elaborated by Tswett.[3] This investigator found that by shaking a perfectly anhydrous petroleum ether or carbon bisulphide solution of the mixed pigments of green plants with an excess of dry calcium carbonate, Inulin or Saccharose, all the pigments will be completely adsorbed by the material with the exception of the carotin, which can be readily washed out of the material with the free solvent. In the case of petroleum ether solutions the green colored mass can now be completely freed from all its pigments by means of petroleum ether containing ten per cent absolute alcohol. If the resulting solution is now shaken with eighty per cent alcohol the petroleum ether layer will contain the chlorophyll pigments and the alcohol layer the xanthophylls. "If to the petroleum ether solution of the mixed pigments

1. Proc. Roy. 72 (1903).
2. Ber. der. Deut. Botan. Gessel. 29, p. 630 (1911).
3. Ber. der. Deut. Botan. Gessel. 24, p. 316 and 384 (1906).

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is added adsorption material only sufficient to destroy the fluorescence, both the carotin and xanthophylls remain in solution," and can be separated by means of a differentiation between the petroleum ether and eighty per cent alcohol, or, "By treating the solution with more adsorption material, after pouring it away from the first, and the xanthophylls then freed from combination with the adsorption material by means of alcoholic petroleum ether." In addition to the above, Tswett made the interesting discovery that the pigments which are adsorbed by the various materials suggested, can to a certain extent displace one another in the adsorbing material. As an example one finds that, "If a petroleum ether solution of the mixed pigments is filtered through a column of adsorption material (such as $CaCO_3$ packed tight in a glass tube) the pigments will be separated from one another from top to bottom in differently colored zones, proportionately to their degree of adsorption." This separation will be complete if a stream of pure solvent is put through the column after the pigment has been adsorbed in the upper part of the column. As stated by Tswett, "Like the rays of light in the spectrum, so the different components of a pigment mixture are actually separated in the $CaCO_3$ column, and may thus be qualitatively estimated." Tswett calls such an experiment a "chromotogramm." He found carbon bisulphide to be one of the most useful solvents for a chromotographic analysis, on account of the brilliant color which all pigments show in this solvent.

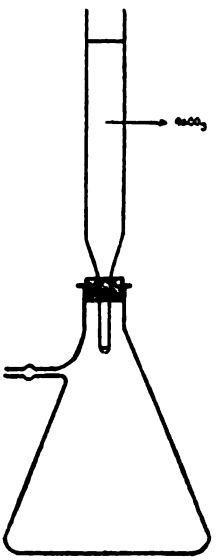

FIGURE I.

In describing the technique for the chromotographic analysis, the author mentions the following essential points. A very finely divided material with not too strong adsorption properties should be used for the adsorbator. ($CaCO_3$ was found to answer these qualifications best.) A glass tube is now prepared 10 to 20 m. m. in diameter and 15 to 20 c. m. long, one end of which is drawn out to a narrow diameter, at which end the opening is fused in a little to form a base for the deposition of the adsorbing material. A small

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piece of cotton is placed in the small end of the tube and the perfectly dry $CaCO_3$ poured in and firmly tamped down to a homogenous texture. The chromotographic apparatus may now be arranged according to Figure I, and the filter flask attached to the suction pump. The $CaCO_3$ is now moistened with a little of the solvent to be used, (this is very necessary) and a certain amount of the liquid which is to be studied poured on the $CaCO_3$. A stream of pure solvent is subsequently established and the different adsorption zones will then spread out and reach their definite maximum differentiation. All unadsorbed substances will be completely washed away and, "Substances which form truly dissociable adsorption compounds with the $CaCO_3$ pass slowly, 'ringwise' through and can be taken up each by itself at the mouth of the tube."

Carotin and Xanthophyll of Green Plants.

After selecting the methods of differentiation and characterization to be applied to the milk fat pigment, it was considered desirable to ascertain whether they were sufficiently characteristic for a complete identification, should the milk fat pigment be found to be either a carotin or a xanthophyll. The following experiment was accordingly carried out.

About fifteen grams of air dry and finely divided alfalfa hay which had a deep green color, was let stand for several days, with shaking, under pure carbon bisulphide. The resulting deep olive brown fluid was concentrated to about 25 c. c. at a low temperature. A glass tube about eight inches long and one-half inch in diameter, the last two inches of which were drawn out to a small opening, was now filled and packed with pure $CaCO_3$, which had been previously dried for two hours at 150° C. The $CaCO_3$ was tamped in a small portion at a time by means of a small cotton wad and a heavy glass rod. The small end of the tube was now inserted through a one hole rubber stopper and fitted tightly into a side neck test tube. The apparatus was then attached to a suction pump. A stream of pure carbon bisulphide was passed through the column. When the $CaCO_3$ had become thoroughly moistened, 2 to 3 c. c. of the alfalfa extract was poured into the top of the column, vigorous suction being maintained all the time. When the extract had passed entirely into the $CaCO_3$ and occupied about one inch of the column, a stream of pure carbon bisulphide was run through. As the pigment passed through it differentiated itself into a number of green and yellow zones, the least

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adsorbed pigment at the bottom of the differentiation having a rose color. The stream of carbon bisulphide was continued until the rose colored solution began to drop from the lower end of the tube. The appearance of the column at this time is shown in Figure II. The stream of carbon bisulphide was now continued until the rose colored zone had entirely passed through.

According to Tswett this zone contained only carotin. The beautiful rose or red orange solution that was obtained was studied as follows: It was first examined in the spectroscope where it showed two distinct bands, and a faint third one. (See Table I.) The carbon bisulphide solution was then evaporated. The residue was on orange yellow solid. A portion of it gave a beautiful blue coloration with concentrated H_2SO_4.

The remainder of the residue was dissolved in hot ninety-five per cent alcohol, and the phystosterol which precipitated out on cooling filtered off. The filtrate was divided into two portions, petroleum ether (b. p. 30-50° C) being added to one and pure carbon bisulphide to the other. On separation of the respective solvents with a little water, the pigment was found quantitatively in the petroleum ether and carbon bisulphide layers respectively. The pigment was again put into alcohol, and in this solvent showed two strong absorption bands and end absorption. (See Table I.)

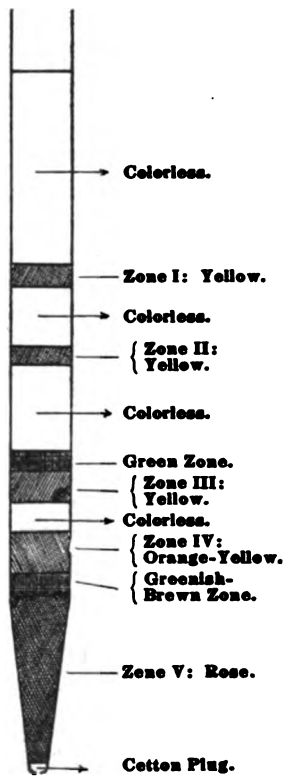

FIGURE II.

Potassium hydroxide was now added to the alcoholic solution and the solution boiled for several hours. The alkaline solution was diluted with three volumes of distilled water and extracted with an equal volume of ether. The ether completely and readily extracted the pigment, showing it to be unsaponifiable. The golden yellow ether solution was washed free from alkali with water and evaporated to dryness. The residue was taken up with alcohol. This solution showed only two bands. (See Table I.)

The alcoholic solution was now tested again for its relative solubility toward petroleum ether (b. p. 30-50° C) and carbon bisul-

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phide. The pigment was again found quantitatively in the petroleum ether and carbon bisulphide. A portion of pigment was again put into alcohol and the solution made strongly alkaline with solid sodium hydroxide. On addition of much sodium chloride to this solution the pigment was not precipitated. The remainder of the pigment was now dissolved in carbon bisulphide, after evaporation of the solution (petroleum ether), and the carbon bisulphide solution filtered through the $CaCO_3$ column again. It passed through unadsorbed as a rose colored zone.

TABLE 1. ABSORPTION (a) BANDS OF ALFALFA CAROTIN

	In CS_2	In C_2H_5OH	In C_2H_5OH (after saponification)
Band I	225-245	256-275	256-277
Band II	259-279	300-320	303-320
Band III	300-320	345-...	.....

(a) Note: It should be noted that all spectroscopic measurements, both this and subsequent ones, were made according to an arbitrary scale which was attached to the spectrometer. This scale was always set to a fixed standard before studying each pigment, the standard being produced by a sodium flame with the spectrometer slit closed to furnish the narrowest possible line. The spectrometer was equipped with a crown glass prism and lenses and had a narrow dispersion.

The study of the alfalfa carotin showed conclusively that its adsorption, spectroscopic and solubility properties were clear and characteristic and were unchanged by boiling in alcoholic potash. It was also shown that the pigment could not be salted out of its sodium alcoholate solution with common salt when the pigment was free from fat. If the solution had contained much soap the pigment would in all probability have been precipitated with the soap in the salting out process. The object of the test was to see whether this was or was not a characteristic test for a comparatively pure carotin. Newbigin [1] claimed to have found a true lipochrome which could be salted out of its alkaline solution.

1. D. Nöel Paton. "Investigations on Life History of the Salmon." 1898, Art. XV. p. 159.

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After studying the carotin pigment, more carbon bisulphide was run through the column shown in Figure II. until the lower green zone was washed out. This was removed from the test tube and the stream of carbon bisulphate continued until the two least adsorbed orange-yellow zones had been washed out. The carbon bisulphide solution of these pigments had a golden yellow color with a faint green tinge. Before the spectroscope the solution showed two chlorophyll bands in the red, and three very clear bands in the blue. (See Table 2.)

The carbon bisulphide was evaporated off and the residue dissolved in hot eighty per cent alcohol, the phytosterol which precipitated on cooling being filtered off. The alcoholic filtrate was now extracted with petroleum ether (b. p. 30-50° C), which took up some green color. This was continued until no more green was extracted. The golden yellow alcoholic xanthophyll solution was examined in the spectroscope. No chlorophyll bands were visible but only two strong bands in the blue and sharp end adsorption.

The alcoholic solution was now saponified with potassium hydroxide and the diluted soap extracted with ether in the usual way. The color immediately went into the ether, the separation being complete with one extraction. After washing free from alkali the ether was evaporated. A portion of this residue as well as a portion of the residue of the solution before saponification, showed a beautiful greenish blue coloration with concentrated H_2SO_4. The remainder when dissolved in alcohol, gave a golden yellow solution showing two bands and end absorption. (See Table 2.)

TABLE 2. ABSORPTION BANDS OF ALFALFA XANTHOPHYLLS

	In CS_2	In C_2H_5OH	In C_2H_5OH (after saponification)
Band I	232-250	265-282	265-286
Band II	273-293	306-326	306-326
Band III	312-330	357-. . .	355-. . .

A little HCl added to a portion of the alcoholic solution gave no blue coloration. Solubility tests on the saponified pigment showed that petroleum ether extracted no color from an alcoholic solution on dilution with a little water, while carbon bisulphide extracted about an equal portion.

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These tests showed conclusively that this pigment belongs to the α group of xanthophylls; and that its adsorption, solubility and spectroscopic properties which are characteristically different from those of carotin, are unaltered by treatment with alcoholic potash.

The remaining xanthophyll pigment was so firmly held in combination with the $CaCO_3$ that carbon bisulphide would not wash it out. A stream of ten per cent alcoholic petroleum ether was therefore run through the column, washing out all the remaining pigments. The resulting solution showed only faint absorption bands, indicating that the pigments noted above, i. e. carotin and xanthophyll α, are the principal yellow pigments of the alfalfa hay.

IDENTIFICATION OF THE PIGMENT OF BUTTER FAT.

With the properties of carotin and xanthophylls well established, attention was next directed to the butter fat pigment. The following experiments were carried out, the results of which are very striking.

Experiment 1.

Fifteen grams of very yellow butter fat from a Jersey cow who was on fresh, green, fall grass was saponified in the usual way with alcoholic potash, taking great care to avoid the presence or formation of the colored alcohol decomposition products, the aldehyde resins. After dilution the soap was extracted with ether. The ethereal extract was washed free from alkali with distilled water and evaporated into ninety-five per cent alcohol. An equal volume (100 c. c.) of petroleum ether (b. p. 30-50° C.) was now added to the alcoholic solution and just enough water to cause a separation of the alcohol and petroleum ether. The golden yellow petroleum ether layer which resulted, contained practically all the color. The alcohol layer was drawn off and extracted with fresh volumes of petroleum ether until only a trace of color went into the petroleum ether layer. All the petroleum ether extracts were then combined and extracted with eighty per cent alcohol. A mere trace of color went into the alcohol. The alcohol solutions were combined.

The Petroleum ether solution.—This contained by far the greatest part of the total pigment. The solution was evaporated quickly at a temperature below 50° C., leaving a red oily residue which instantly dissolved in carbon bisulphide with a deep red orange color. After adjusting the concentration for the 10 m. m. cell, so that the bands

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were all plainly visible, this solution showed three distinct absorption bands. (See Table 3.)

The carbon bisulphide solution was evaporated to dryness at the lowest possible temperature, the residue taken up in 50 c. c. of hot ninety-five per cent alcohol and hot one per cent digitonin in ninety per cent alcohol added until no more precipitate came down. The digitonin-cholesteride was filtered off, the filtrate evaporated to dryness, and the residue dissolved once more in carbon bisulphide. The solution still showed the three bands. On evaporation it left a golden yellow oil which solidified on cooling to a reddish yellow salve. Concentrated H_2SO_4 added to the residue gave a blue green color which slowly changed to a purple color.

The alcoholic solution.—This was evaporated to dryness. When very concentrated it showed a little color, and the carbon bisulphide solution of the residue had a light orange color when it had a volume of 1½ c. c. When viewed in a 25 m. m. cell this solution showed three absorption bands. (See Table 3.)

TABLE 3. ABSORPTION BANDS OF BUTTERFAT PIGMENTS.

	Petroleum ether soluble pigment In CS_2 solution		Alcohol soluble pigment in CS_2 solution
	Crude	Free from Cholesterol	
Band I	222-240	224-242	229-247
Band II	257-276	260-277	269-288
Band III	299-319	301-316	309-328

The striking results of this experiment were the remarkable similarity of the solubility and spectroscopic properties of the main butter fat pigment to carotin, and the indications of a secondary minor constituent of the butter fat pigment, whose solubility and spectroscopic properties were strikingly similar to xanthophyll.

It at once became evident that should these observations be confirmed, it would be not only profitable, but essential to ascertain whether the presence of secondary xanthophyll-like pigments is normal to butter fat under other conditions of coloration, such as in light colored butter fat, colostrum butter fat and other conditions.

That the presence of a secondary pigment in the fat under investigation was confirmed and its character more clearly identified is shown by the following experiment.

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Experiment 2.

Fifteen grams of the fat was treated as in Experiment 1. In addition the carbon bisulphide solution of the unsaponifiable ether extract was filtered through a column of $CaCO_3$ in a manner identical with the chromotographic experiment with alfalfa hay. As far as could be detected with the eye all the pigment passed quite rapidly through as an unadsorbed rose colored zone, which spread out considerably in its passage through the column but showed no differentiation into zones. The absorption bands of the filtered pigment were identical with the bands of carotin.

After the carbon bisulphide had washed out all the pigment and was passing through colorless, a stream of petroleum ether containing ten per cent alcohol was run through the column. As it passed through it gathered a zone of yellow color, leaving the column pure white. This pigment was collected at the mouth of the tube, its solution evaporated, and the residue dissolved in carbon bisulphide. The light-orange colored solution showed two strong absorption bands and a third fainter one.

The carbon bisulphide solution of the main pigment was now evaporated to dryness and the residue dissolved in ninety-five per cent alcohol. The alcohol was diluted with water to an eighty to ninety per cent solution and extracted with petroleum ether (b. p. 30-50° C). The bulk of the pigment went into the petroleum ether and a second extraction with fresh petroleum ether took out still more pigment. A third extraction with fresh petroleum ether, however, left the alcohol layer considerably more colored than the petroleum ether layer. The alcohol layer was now evaporated and the residue dissolved in carbon bisulphide, giving an orange yellow solution which showed three strong absorption bands. There seemed to be three or four times as much of this pigment as of the xanthophyll which had been adsorbed by the $CaCO_3$ in the chromotogramm, and together they probably amounted to ten per cent of the total pigment.

All the xanthophyll pigments were now combined (they were all in carbon bisulphide solution) and the resulting solution analyzed by means of a chromotogramm. As the orange-yellow solution was washed through the column by a stream of carbon bisulphide it took on the appearance as shown in Figure III. Zones two and three were collected together, and showed three absorption bands. (See Table 4.) Their solution was evaporated and the residue dissolved in petroleum

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ether. Only a part of the residue would dissolve readily, and the remainder only on addition of a little absolute alcohol. Eighty per cent alcohol extracted a large part of the color from the alcoholic petroleum ether solution. The portion which remained in the petroleum ether was transferred to carbon bisulphide in which it showed three absorption bands. (See Table 4.) The portion extracted by the eighty per cent alcohol was also transferred to carbon bisulphide. The latter solution showed three absorption bands but the third was faint and was not measured. (See Table 4.)

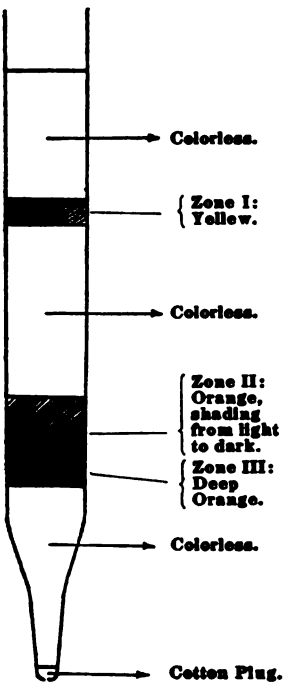

FIGURE III.

Alcoholic solutions of both portions of pigment showed no color change on the addition of a little concentrated HCl. There was also no effect on the absorption bands.

Zone I of the chromotogramm was of a pure yellow color. It was completely adsorbed by the $CaCO_3$ with respect to CS_2 and was evidently the same pigment which had been adsorbed in the first chromotogramm of the combined carotin and xanthophyll-like pigments. A stream of alcoholic petroleum ether readily washed it out of the column as it did in the first chromotogramm. In carbon bisulphide solution the pigment had a light orange color, and showed two brilliant absorption bands and a third fainter one. (See Table 4.)

The pigment showed the three bands in alcoholic solution as well as in carbon bisulphide. When in alcohol, it gave no color reaction with a little concentrated HCl, and there was also no immediate effect upon either the intensity or position of the absorption bands. In the solid state this pigment gave a transient greenish-blue color with concentrated H_2SO_4.

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For the sake of comparison with the carotin bands of the alfalfa hay, the alcoholic solution of the main butter fat pigment was examined in the spectroscope. The results are given in Table 5.

TABLE 4. ABSORPTION BANDS OF BUTTER FAT XANTHOPHYLLS SHOWN IN FIGURE III.

	Zone I. CS_2 solution	Zones II & III C_2H_5OH solution	Zones II & III	
			(P. ether sol. Part) In CS_2	(Alcohol sol. Part) In CS_2
Band I	232-249	263-280	230-249	231-250
Band II	271-288	306-325	268-289	272-240
Band III	313-330	355-. . .	312-330	Not measured

TABLE 5. COMPARISON OF BANDS OF CAROTIN OF ALFALFA AND BUTTERFAT. (C_2H_5OH SOLUTION).

	Alfalfa Carotin	Butterfat Carotin
Band I	256-275	256-274
Band II	300-320	298-312
Band III	345-. . .	345-. . .

The result of this experiment was not only to confirm the remarkable similarity of the main butter fat pigment of this particular fat to the carotin of alfalfa hay, but also to confirm the presence of a secondary constituent practically identical with the xanthophylls. In addition it was found that these xanthophylls, like the xanthophylls of alfalfa hay, could be chromotographically separated into three constituents. The two main constituents seemed to be closely related to carotin in adsorption properties so that their presence could not be detected in the presence of a large amount of carotin until first separated from the main pigment with the aid of their relatively greater solubility in alcohol with respect to petroleum ether. The third constituent of the secondary group was more nearly related to a true xanthophyll in all its properties, including its adsorption by $CaCO_3$. When classified with respect to the action of their alcoholic solutions toward a little concentrated HCl all the xanthophyll pigments apparently belong to the group which Tswett calls α xanthophylls.

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STANDARDIZATION OF ABSORPTION BANDS OF CAROTIN AND XANTHOPHYLLS.

Before proceeding further it may be well to make a few statements in regard to the spectroscopic properties of the various pigments which have been under consideration and which are still to be considered. It will be obvious that measurements of absorption bands could not be made in this work with the accuracy attained by Willstätter and his collaborators who used solutions of standard strength and a spectroscope of great exactness. Small differences in measurement among xanthophylls or between carotins should accordingly be disregarded. It was merely attempted in every case to secure solutions of such concentration that the bands were of as nearly the same intensity as could be detected with the eye before making measurements. It was not always possible to use the same thickness of cell to secure the required intensity of the bands, small amounts of pigment naturally requiring a greater depth of solution than large amounts.

In order to have standard spectroscopic properties for future comparison, carotin and xanthophylls were extracted from the carrot, and a careful study made of the spectroscopic properties of each pigment.

A grated carrot was boiled in water for about two hours, the water squeezed out of the pulp and the pulp dried on the steam bath. It was pulverized and extracted with ether in a Soxhlet extractor until colorless. The ether was evaporated into ninety-five per cent alcohol at a low temperature. The resulting solution was diluted with a little water to eighty per cent and the pigment carefully separated between petroleum ether (b. p. 30-50° C) and the eighty per cent alcohol. Both portions of the pigment were carefully transferred to pure carbon bisulphide and the solutions adjusted for the 10 m. m. cell until all bands were distinct and as nearly as possible of equal intensity. With the spectrometer set at the sodium line standard the positions of the absorption bands were standardized. The color of the solutions was measured also, by means of the Lovibond tintometer. (See Figure V.)

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This data is given in Table 6.

TABLE 6. SPECTROSCOPIC STANDARD OF CAROTIN AND XANTHOPHYLLIS. (FROM THE CARROT.)

Pigment	Layer	Color			Absorption Bands		
		Yellow	Red	Light	Band I	Band II	Band III
In CS_2							
Carotin	10 m.m	26.0	5.5	1.0	225-242	261-278	301-319
Xanthophyll	10 m.m	17.0	4.4	0.5	233-253	272-291	312-330
In C_2H_5OH							
Carotin	10 m.m	....	...	...	257-275	303-318	345-364
Xanthophyll	10 m.m	....	...	...	263-280	305-325	355-. ..

It will be noticed that the relative position of the bands of carotin and xanthophylls is more characteristic in carbon bisulphide than in alcohol. On this account, nearly all subsequent spectroscopic studies were made in carbon bisulphide solution.

It will be noticed that both pigments showed three bands. The difference in the color of the carbon bisulphide solutions of the carotin and xanthophyll pigments when showing bands of equal intensity is also especially noteworthy. To the eye, the carotin solutions are a deep red orange while the xanthophyll solutions are a much purer orange color. It is worthy of mention that this characteristic difference was so noticeable throughout all this study, that in all cases the color of the carbon bisulphide solution of unknown pigments was recorded in connection with the measuring of the absorption bands.

CHARACTER OF THE PIGMENTS IN DIFFERENT BUTTER FATS.

It was stated previous to the confirmation of the presence of secondary xanthophyll-like pigments in the particular butter fat studied, that if the presence of these pigments could be confirmed it would necessary to establish such a fact as either characteristic of all butter fat or as incidental only to the particular fat studied.

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A wide variety of fats of different color, from different breeds of cows and produced under widely different conditions of feeding, etc., were available for this study. The technique of these experiments was identical with that used for the high colored fat from the Jersey cow on grass recorded in Experiments 1 and 2 above.

The Pigments of Light Colored Fats.—The character of the pigment in three light colored fats was tested. The fats represent three subsequent periods in a feeding experiment of a pure bred Ayrshire cow in which the color was practically eliminated from the butter fat. (See Table 13 of feeding experiments for record of this experiment.) The color of the fat and the character of the pigments are shown in the following Table 7.

TABLE No. 7.

Color of Fat		Character of Pigments.
Yellow	Red	
6.0	1.0	Petroleum ether quant. extracted cholesterol-free pigment from 80 per cent alcohol. Solubility test showed carotin and xanthophylls.
2.5	0.7	Chromotogramm of xanthophylls showed adsorbed yellow constituent and unadsorbed orange zone.
1.4	0.5	Pigment from 240 gm. fat had color of 35 yellow, 1.0 red. Solubility test showed both carotin and xanthophylls, as did also absorption bands.

The Pigments of Butter Fat After Carrot Feeding.—The fat tested was taken during a carrot feeding experiment with the same cow used in the above experiments. This feeding experiment directly followed the third period of very light colored fat. The color of the fat was 28 yellow and 1.4 red. Solubility tests on the pigment showed carotin and a very small amount of xanthophyll. The petroleum ether soluble part of the pigment gave a CS_2 chromotogramm of a single unadsorbed rose colored zone. The filtered pigment showed three well defined absorption bands. I, 225-245; II, 263-283; III, 301-320.

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The Pigments of the Fat from Colostrum Milk.—It is a well known fact that the first milk drawn after parturition always has a high yellow color. It is not generally known, however, that this high color is usually due [1] entirely to the suspended fat globules. We have many times observed, not only in connection with this study but also in connection with numerous studies dealing with the chemical composition of milk, that when the fat is entirely removed from colostrum milk the skim milk has the appearance of ordinary skim milk, and the butter and the rendered fat have a depth of color which is never equaled at any subsequent stage of the lactation period. This characteristic of colostrum milk is common to all breeds of cows, and the high color of the fat continues in cows of all breeds for a short time after parturition and then gradually falls off. Table 8 gives the color of the milk fat of several cows shortly after parturition and again a week or two later. The color readings are the Lovibond tintometer readings of a one-inch layer of melted, rendered fat.

TABLE NO. 8. COLOR OF THE FAT OF COLOSTRUM MILK.

Cow No.	Breed	Roughage fed.	Days after Parturition.	Color		
				Yellow	Red	Light
301	Ayrshire	Alfalfa(a)	4	78.0	3.5	1.0
301	Ayrshire	Alfalfa	26	71.0	1.5	0.5
300	Ayrshire	Alfalfa	4	71.0	3.5	1.0
300	Ayrshire	Alfalfa	20	68.0	2.8	1.0
2	Jersey	Alfalfa	13	68.0	2.6	0.5
2	Jersey	Alfalfa	22	57.0	2.5	0.5
2	Jersey	Alfalfa	2	54.0	4.3	1.0
2b	Jersey	Alfalfa	20	50.0	2.5	1.0
20	Jersey	Alfalfa	2	47.0	4.8	1.0
20	Jersey	Alfalfa	25	47.0	2.0	0.5
206	Holstein	Alfalfa	1	50.0	4.7	0.3
206	Holstein	Alfalfa	5	54.0	2.0	0.2

(a) The Alfalfa hay was rich in carotin and xanthophylls.
(b) Second sample taken after next parturition.

This phenomenon at once offered the interesting problem of the relation of the colostrum pigment to the pigment of normal butter. A close study was accordingly made of the pigment of the fat from the first milk drawn after parturition. The cow selected

1. Colostrum milk is occasionally contaminated with blood.

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for study was a pure bred Holstein. The fat tested had a very high color; a one inch layer gave a reading of 64 yellow, 5.0 red and 1.0 light in the Lovibond tintometer.

The unsaponifiable pigment and impurities from fifteen grams of the fat had a golden yellow color in ether and in carbon bisulphide a blood red color. This solution was analysed chromotographically. The entire pigment passed through unadsorbed as a red orange or rose colored zone, leaving no adsorbed zones and no pigment behind in the $CaCO_3$ which could be washed out with ten per cent alcoholic petroleum ether. The filtered solution showed two strong absorption bands and a third faint one. (See Table 9.)

The pigment was now analyzed according to its proportionate solubility in petroleum ether (b. p. 30-50° C) and eighty per cent alcohol, and was thus divided into two portions, a major portion extracted by the petroleum ether and a very minor portion which the petroleum ether would not extract from the eighty per cent alcohol.

The carotin-like pigment thus obtained was freed from cholesterol by the digitonin method and its bands again measured in carbon bisulphide solution. (See Table 9.)

The residue from this solution gave a beautiful transient blue color with concentrated H_2SO_4 and a very transient blue-green color with concentrated HNO_3.

The eighty per cent alcohol soluble pigment showed three absorption bands in carbon bisulphide solution, the first two being a little more intense than the third. (See Table 9.) While in this solution the pigment was analyzed by means of a chromotogramm and showed two zones, a primary little adsorbed zone of orange color, and a secondary more adsorbed zone of yellow color. Hydrochloric acid gave no color reaction with the alcoholic solution of the pigment of either zone.

TABLE 9. ABSORPTION BANDS OF PIGMENTS OF COLOSTRUM MILK FAT.

	Combined Pigment (CS_2 Solution)	Carotin (CS_2 Solution)	Xanthophylls	
			(CS_2 Sol.)	(C_2H_5OH Sol.)
Band I	223–240	224–242	232–249	264-281
Band II	260–278	259–278	272–291	306–326
Band III	300–320	302–319	312–330	356–. . .

Crystalline Form of Carotin From Butter Fat.—The great concentration of pigment in the fat from colostrum milk seemed to offer an excellent opportunity to at least attempt the isolation of the pig-

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ment in crystalline form. The pigment was isolated in the usual way from forty grams of very high colored colostrum fat from a Jersey cow. Great care was taken during the isolation to avoid aldehyde resin pigments. This was successful. The ether solution of the unsaponifiable substances was evaporated at 35° C. and the residue dissolved at once in carbon bisulphide. This solution, which had a blood red color, was concentrated to 2 c. cm. volume at a low temperature and an excess of cold absolute alcohol added. There was no immediate crystallization, but after standing several days there were deposited a number of yellow crystals. These were at first thought to be crystals of the pigment, for similar crystals were obtained in the same manner from the corpus luteum pigment. The crystals were perfectly formed double pyramidal forms, but proved to be crystals of sulphur which evidently arose from the carbon bisulphide used. A further attempt at crystallization of the pigment was abandoned. It undoubtedly would prove successful if sufficient pure material could be obtained.

THE RELATION BETWEEN THE COLOR OF THE MILK FAT AND THE FOOD OF THE COW.

General observation for no doubt hundreds of years, at least, ever since butter has become of importance in the diet of man, has shown that green feeds of all kinds, especially fresh green grass greatly increase the color of butter fat. Other feeds, such as carrots, beets and yellow corn have been said to have the same effect. It has never been the subject of a scientific investigation however, to show just what relation exists between the food of the cow and the color of the milk fat. With a chemical relation established between the milk fat pigments and carotin and xanthophylls, the relation of the color of the milk fat to the food seems to be readily explained on the ground that the foods that have been observed to cause the highest colored butter, namely, green foods, carrots, etc. are those which are especially rich in carotin and xanthophylls, particularly carotin, as in the case of the carrots. Indeed we can go still further and definitely state that these pigments must be abundantly present in the food before the milk fat will show a high color. as will be demonstrated by the experiments which are about to be reported.

Character of the Yellow Pigments of the Common Cattle Feeds.— The chemical study of the yellow pigment of milk fat, shows that its principal constituent belongs to the hydrocarbon or carotin group of pigments, although it also contains xanthophylls as a very minor secondary constituent. It accordingly became necessary to study the

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nature of the pigments of various common cattle foods before conducting any feeding experiments to prove that there is a direct relation between the presence of carotin in the butter fat and the presence of carotin in the food. It was at first merely sought to show the presence or absence of unsaponifiable yellow pigments in various foods, by extracting them with hot alcohol, saponifying the extract with potassium hydroxide and extracting the pigment from the soap with ether. From this standpoint, it was found that among the grains and concentrates, wheat bran, linseed meal, dried brewer's grains and cottonseed meal all showed the presence of small amounts of unsaponifiable yellow pigments, while white corn was found to be the only common grain absolutely free from such pigments. Yellow corn was of course found to be rich in unsaponifiable yellow pigments. Some roughages such as corn silage and cottonseed hulls were found to contain small amounts of unsaponifiable pigments, while wheat straw and oat straw were practically free from them. The hays were found to be the most variable of all feeds. All green hays,[1] such as alfalfa, first-class clover and the very best timothy were found to contain considerable amounts of unsaponifiable yellow pigments, the amounts varying with the greenness of the hay. Bleached hays, whether timothy, clover, or alfalfa were more or less free from unsaponifiable yellow pigments.

Some feeds were investigated more particularly with a purpose of showing the character of the unsaponifiable yellow pigments. Without reporting the experimental details in all cases, but merely stating that the methods of analysis were spectroscopic, chromotographic, and solubility methods, the following results were obtained.

Cotton Seed Meal and Cotton Seed Hulls.—It was found that the unsaponifiable yellow pigments of cottonseed meal and cottonseed hulls were due entirely to the oil they contain; and further that this oil which is known to be characterized by its yellow color contains equal proportions of carotin and xanthophyll. The carotin is the usual one met in other places, while the xanthophyll is made up of at least five different constituents according to their adsorption properties. The chief xanthophyll is not adsorbed to any extent by $CaCO_3$ from its carbon bisulphide solution, and in this solvent shows absorption bands of characteristic position, shifted considerably toward the blue from the normal xanthophyll bands. In CS_2 the bands measured: I, 238-260; II, 285-303; III, 355—. The remainder of the xanthophylls were so firmly held by the $CaCO_3$ that they could not be readily

1. By green hay is meant hay that has been cured under such conditions that it still retains a large part of the green color which characterizes its uncut condition. The green alfalfa hay referred to throughout this paper was western cured alfalfa which had a remarkably bright green color.

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washed out with a stream of carbon bisulphide. They were all washed out however, by a one per cent alcoholic petroleum ether solution.

Bleached Alfalfa Hay.— This hay was quite free from green stalks and as it had been found palatable to the cows it was of special interest as an experimental roughage for non-pigmented feeding studies. A selected sample of the hay was ground up fine, and extracted with ten per cent alcoholic petroleum ether. The light green colored extract showed no indication of either carotin or xanthophyll when analyzed by means of the chromotogramm, and no yellow pigment was extracted from its alcoholic solution by petroleum ether or by carbon bisulphide.

Yellow Corn.—The unsaponifiable yellow pigment of yellow corn is in all probability in the oil. It was found to be composed of two constituents, the largest part of which is a xanthophyll-like pigment, showing absorption bands in alcoholic and carbon bisulphide solutions lying close to the normal xanthophyll bands. In CS_2 the bands measured: I, 229-251; II, 274-291; III, not measured. It was not adsorbed from either petroleum ether or carbon bisulphide by $CaCO_3$ but passed through as a yellow or orange zone. Its carbon bisulphide solutions were orange colored. Petroleum ether readily extracted the pigment from its concentrated eighty per cent alcoholic solution, but it could be completely re-extracted from its petroleum ether solution by fresh eighty per cent alcohol. In this respect it differed from any xanthophyll-like pigment yet investigated. The pigment was more soluble in carbon bisulphide than in eighty per cent alcohol and in this respect favored carotin. On warming its alcoholic solution containing a little concentrated hydrochloric acid, the color of the solution changed to a light greenish blue, with the fading of the absorption bands. The minor constituent of the corn pigment had the spectroscopic, solubility and adsorption properties of carotin.

The Carrot.—It was planned to conduct some feeding experiments with carrots, and a special study of its relative proportion of carotin and xanthophyll was accordingly considered advisable. A large well-colored carrot was scraped, chopped fine, and boiled in water for one hour. The softened tissue was poured onto cheesecloth and the water squeezed out of the pulp. The pulp was pressed through a wire gauze, dried and powdered. The meal was extracted with carbon bisulphide and a portion of the blood red solution analyzed by means of a chromotogramm. By far the greatest part of the pigment passed through unadsorbed as a rose colored zone, leaving a small amount of adsorbed pigment in the column which was not differentiated into zones but which was readily washed out with alco-

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holic petroleum ether. The proportion of xanthophyll to the entire pigment in this test was very small, not over three or four per cent.

The rose colored carotin solution showed three beautiful absorption bands, all of equal intensity. The measurements of the bands were completely in accord with the standards given in Table 6.

The remainder of the carbon bisulphide extract of the dried carrot meal was evaporated into alcohol. The alcohol was diluted with water to an eighty per cent solution and extracted with petroleum ether (b. p. 30°-50° C) until no more color was extracted. The alcoholic solution of the xanthophylls which remained was combined with the solution of the xanthophylls washed out of the chromotogramm with alcoholic petroleum ether. The combined solutions were evaporated to dryness and the residue dissolved in a small amount of carbon bisulphide. This solution showed three absorption bands and end absorption. The measurements were the same as given in Table No. 6.

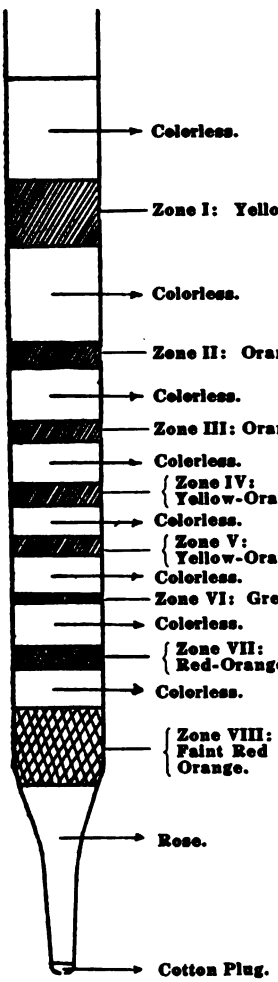

FIGURE IV.

This carbon bisulphide solution was orange colored when concentrated and gave the chromotogramm shown in figure IV. There was a small amount of carotin pigment present which being unadsorbed was readily washed out and caught at the lower end of the tube. Zone VIII was also only slightly adsorbed and was washed out by a stream of carbon bisulphide. When this zone was washed out the suction was stopped. The $CaCO_3$ tinted zones were then removed separately from the column with the aid of a knife; the CS_2 was drawn off in each case at a low temperature; and the pigment washed out with ninety-five per cent alcohol.

Zone II was found to contain the most pigment, judged from the color of its alcoholic solution, with the lower zones about the same with the exception of Zone V which had very little pigment. Zone I contained considerably more pigment than was indicated by the color of its zone in the chromotogramm. The spectroscopic properties of all the xanthophyll constituents were studied with respect to the normal xanthophyll bands. The results

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are shown in Table 10. The observations were not made by observing all the pigments in the same volume but the volumes were adjusted in each case to give the best possible bands.

It will be noticed that with the exception of Zone I, all the xanthophylls showed the three normal xanthophyll bands to some degree of intensity. Similarly hydrochloric acid had no effect on any of these solutions or on the bands. Nitric acid had the same effect on the pigments of Zones II, VII, and VIII causing the solutions to fade with the disappearance of the bands. The effect of nitric acid on the pigments of Zones III and IV was somewhat different. In these two cases a fourth well-developed band appeared before the solution lost its color and the three normal bands faded. The pigment of Zone I was entirely different from any of the others. The normal first band of the xanthophylls was entirely missing and the alcoholic solution turned a distinct bluish green on the addition of a little concentrated hydrochloric acid. This color persisted for 24 hours, long after the absorption bands had disappeared. This was evidently the xanthophyll *B* of Tswett and the xanthophyll *B* of C. A. Schunck.

TABLE NO. 10. SPECTROSCOPIC PROPERTIES OF THE XANTHOPHYLLS OF THE CARROT.

Zone	Band I	Band II	Band III	End Absorption
I	Missing	Good	Good	Very faint
II	Fair	Fair	Fair	None
III	Very strong	Very strong	Weak	None
IV	Strong	Very strong	Faint	None
V	Very faint	Good	Good	None
VII	Very strong	Very strong	Very faint	None
VIII	Good	Good	Very faint	Very faint

THE FEEDING EXPERIMENTS.

The foregoing studies indicated that the foods best adapted for non-pigmented rations were bleached hays and cottonseed hulls for roughages and white corn and cottonseed meal for grains. To study the variation in the color of butter fat, the ration of various cows was changed to one containing the smallest possible amounts of carotin and xanthophylls, and the butter fat studied colorimetrically during this time. The procedure for the butter fat was in each case as follows: The milk of one or two milkings was separated by means of a centrifugal hand separator and the cream churned by hand in

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quart bottles. The butter thus obtained was rendered at a temperature of 50° to 60° C. and the rendered fat filtered. The pure filtered fat was analyzed colorimetrically by means of the Lovibond tintometer and its standard color glasses. The color of the fat was always compared in one-inch layer. The Lovibond tintometer is shown in Figure V.

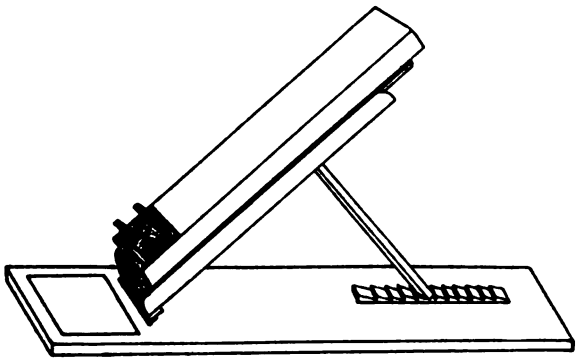

FIGURE V.

The solution (in the present case melted butter fat) whose color is to be measured is placed in a cell with glass ends (one inch apart in all this work), and the color matched by standard color glasses of various units of yellow, red or blue, and the color of the solution read by adding together the various glasses of color used to match the unknown color. Melted butter fat having an orange tint requires only yellow and red to match its color. All readings are made with the instrument pointing towards the daylight (not sunlight). The instrument is quite sensitive towards the yellow glasses below 25 units of yellow but the sensitiveness decreases considerably above 40 units of yellow. In other words, it is possible to match the exact color of an "unknown" much more closely when its color is below 30 to 35 units of yellow than when its color is above this value. In a great many cases, and this nearly always applies to butter fat, it is possible to match the tint of the fat but the color of the fat is more brilliant than that of the combined standard glasses. In this case an exact match can be obtained by "damping down" the butter fat color by inserting in front of it equal units of the three colors, yellow, red and blue, and recording this as "light."

Before reporting the data dealing with the variation in the color of the butter fat it may be possible to convey some idea of what the various colors mean when applied to butter fat by stating that rendered "June" butter in the one-inch cell will give a color of from 80 to 60 units of yellow. Color readings between 45 and 25 units of

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yellow would accordingly indicate a fairly well colored to light colored butter, between 20 and 8 units of yellow would be called light to very light colored butter, while below these limits ranging down to 1 or 2 units of yellow would be called white to "dead" white, especially if the fat was still in the form of butter.

Experiment 1.

The ration of Cow No. 57, a pure bred Jersey, was changed from a ration rich in carotin and xanthophylls to a ration containing a very small amount of these pigments. The ration rich in carotin and xanthophylls consisted of alfalfa hay and yellow corn. The ration poor in these pigments was composed of bleached clover hay and white corn. The results of the experiment are shown in Table 11.

The change from a ration rich in carotin and xanthophylls to one poor in these pigments caused the color of the butter fat to drop from 43 units of yellow to 8.5 units of yellow, from a well colored to a very light colored fat. This change of color was very gradual and required 29 days. It should be stated, however, that the cow did not relish her non-pigmented ration. She lost weight regularly, and her milk production fell off a great deal. It was apparent that the animal was drawing heavily during this entire period from a storage of pigment in her body. It will be shown in the subsequent papers of this series that in this experiment the blood and also the body fat were supplying the pigments for the milk fat.

It may be stated that a slow lowering of the color of the milk fat, such as took place in this experiment, would be normal for all Jersey cows whose ration is changed to an unpalatable, non-pigmented one like that used in this experiment. The explanation for this is found in the high color of the body fat of this breed of cows. We therefore have here a clear explanation of why Jersey cows will sometimes apparently give yellow milk fat during the winter months when their food is almost or entirely lacking in carotin and xanthophylls. Under these conditions if the body fat is called upon to supplement the digestion products of the food in the production of milk fat at the same time the blood serum storage of pigments is being drawn upon, it is clear that the reduction in color of the milk fat will be very gradual, as in the case of Cow No. 57, and a complete elimination of color may require a long period of time.

Continuing the discussion of the experiment it is seen that when the color of the milk fat had dropped to 8.5 units of yellow the white corn in the ration was replaced by mixed corn, white and yellow

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(mostly yellow). Later this was replaced by yellow corn entirely. The roughage remained the same, i. e. bleached clover hay. The table shows that yellow corn had no effect whatever upon the color of the milk fat. There seemed to be a very slight effect at first but the color of the fat soon dropped back to 8.0 units of yellow.

TABLE NO. 10. THE EFFECT OF A CAROTIN AND XANTHOPHYLL-FREE RATION ON THE COLOR OF MILK FAT. JERSEY COW NO. 57.

Date	Pounds hay per day	Pounds grain fed per day		Color of butter fat.		
				Yellow	Red	Light
1912		Corn	Mixture			
Mar. 1	15(a)		8	43.0	2.0	0.2
2	15(b)		8			
3	15	4 white	4			
4	15	4 white	4			
5	15	4 white	4			
6	15	8 white				
7	15	8 white		33.0	2.0	0.2
8	15	8 white				
9	15	8 white		29.0	1.7	0.2
10	15	8 white				
11	15	8 white		33.0	1.8	0.2
12	15	8 white		26.0	1.6	0.2
13	15	8 white		26.0	1.7	0.2
14	15	8 white		22.0	2.0	0.2
15	15	8 white		22.0	2.0	0.2
16	15	8 white		21.0	1.8	0.2
17	15	8 white				
18	15	8 white		18.0	1.7	0.2
19	15	8 white		19.0	1.6	0.2
20	15	8 white		18.0	1.6	0.2
21	15	8 white				
22	15	8 white		12.0	1.6	0.2
23	15	8 white				
24	7	10 white				
25	7	10 white		11.0	1.6	0.2
26	7	10 white		11.0	1.6	0.2
27	7	10 white		10.0	1.5	0.1
28	7	10 white		10.0	1.5	0.1
29	7	10 white		8.5	1.8	0.2
30	7	10 white		9.0	1.7	0.2
31	7	10 mixed		10.0	1.8	0.2

(a) Alfalfa.
(b) Clover—Mar. 2 to Mar. 31.

The 7 lbs. of clover hay was now replaced by 10 lbs. of alfalfa hay rich in carotin and xanthophylls. The effect on the color of the

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TABLE 11 (CONTINUED). EFFECT OF FEEDING YELLOW CORN TO A COW GIVING LOW COLORED MILK FAT. JERSEY COW NO. 57.

Date	Pounds hay per day	Pounds grain fed per day	Color of butter fat		
			Yellow	Red	Light
1912		Corn			
Apr. 1	7(a)	10 mixed	8.5	1.7	0.2
2	7	10 mixed	8.0	2.1	0.2
3	7	10 mixed	8.0	1.9	0.2
4	7	10 mixed	12.0	1.5	0.2
5	7	10 mixed	12.0	1.7	0.2
6	7	10 mixed	11.0	1.8	0.2
7	7	10 yellow			
8	7	10 yellow	11.0	1.8	0.2
9	7	10 yellow	8.0	1.7	0.2
10	7	10 yellow	9.0	1.7	0.2
11	10(b)	10 yellow	10.0	1.5	0.2
12	10	10 yellow	12.0	1.6	0.2
13	10	10 yellow	15.0	1.6	0.2
14	10	10 yellow	20.0	1.6	0.2
15	10	10 yellow	33.0	1.7	0.2
16	10	10 yellow	36.0	1.6	0.2
17	10	10 yellow	38.0	1.6	0.2
18	10	10 yellow	43.0	1.6	0.2
19	10	10 yellow	45.0	2.0	0.5
21	10	10 yellow	43.0	1.8	0.5
22	10	10 yellow	43.0	1.7	0.5
23	10(c)	10 yellow	46.0	1.7	0.5
24	10	10 yellow	40.0	1.8	0.5
25	10	10 yellow	43.0	1.8	0.5
27	10	10 yellow	52.0	2.0	0.5
30	10	10 yellow	57.0	2.1	0.5
May 2	10	10 yellow	64.0	2.1	0.5
4	10	10 yellow	64.0	2.2	0.5
5-10(d)	Pasture only		80.0	2.5	0.5

(a) Clover—Apr. 1-10.
(b) Alfalfa—Apr. 11-22.
(c) Alfalfa and pasture—Apr. 23-May 10.
(d) Sample taken May 10.

milk fat was immediate. At the end of seven days the color had increased to 43 units of yellow, the maximum supplied by this roughage. Later the ration was supplemented by some fresh pasture grass. The color of the milk fat increased to 64 units of yellow. Still later the cow was turned out to pasture alone. The color then reached the maximum we have observed, i. e. 80 units of yellow.

Experiment No. 2.

This experiment was conducted with Cow No. 301, a pure bred Ayrshire. A short time previous to the experiment here reported this

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cow was in very poor flesh resulting from a feeding experiment in which she was heavily underfed. Her ration during her underfeeding was composed of alfalfa hay rich in carotin and xanthophylls and a grain mixture of corn, bran and linseed meal. At the end of the underfeeding experiment the ration was changed to bleached alfalfa hay, the pigmentation of which was reported above, and white corn and cottonseed meal. Enough of this ration was given to bring the cow back to normal feeding conditions. At the end of this time the experiment here reported was begun. The results are given in Table 12.

At the end of ten days the color of the milk fat had dropped to 9 units of yellow. When it was apparent after a week's further trial that the color had reached at least a temporary minimum value for this ration, the grain was changed to yellow corn entirely. This was finally increased to 12 lbs. per day. The result was very clearly in accord with that of Experiment No. 1, showing that yellow corn is not a source of pigment for the milk fat of dairy cows.

TABLE No. 12. EFFECT OF NON-PIGMENTED RATION AND A RATION CONTAINING YELLOW CORN UPON THE COLOR OF MILK FAT. AYRSHIRE COW No. 301.

Date of sample	Pounds alfalfa hay	Pounds corn	Pounds cotton-seed meal	Color of butter fat	
				Yellow	Red
1912					
October 3	16	6(a)	2	27.0	1.7
13	16	6	2	9.0	1.7
17	16	6	2	6.0	1.5
21	16	6	2	9.0	1.2
24	16	8(b)		7.5	1.2
25	16	8		9.0	1.5
26	16	8		10.0	1.5
27	16	8		9.0	1.5
28	16	8		9.0	1.5
29	16	8		10.0	1.5
30	16	8		8.5	1.2
31	16	8		10.0	1.2
November 1	16	12		9.5	1.2
2	16	12		10.0	1.2
3	16	12		8.5	1.2
4	16	12		8.0	1.2

(a) Oct. 3-21, white corn.
(b) Oct. 24-Nov. 4, yellow corn.

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Experiment No. 3.

This feeding experiment was conducted with the same cow as the preceding experiment and immediately followed that experiment.

It seemed very probable that the reason the color of the milk fat in Experiments 1 and 2 could not be lowered more than the uniform figure found in both experiments, i. e., about 8 units of yellow, was due to the fact that the ration was supplying a small amount of pigment and also to the fact that the normal storage, that in the blood serum, had not been exhausted. It seemed reasonable to suppose, therefore, that if the first factor was eliminated at the outset, the second would necessarily also be eliminated if the experiment was continued for a sufficient length of time. The experiment here reported was for the purpose of testing the validity of this supposition, and also for the purpose of ascertaining to how low a point the color of the milk fat could be reduced. The ration chosen was one which would supply the least amount of carotin and xanthophylls. It was composed of cottonseed meal and cottonseed hulls. The results of the experiment are given in Table 13.

The supposition stated above was fully borne out by the long continued feeding of a practically non-pigmented ration. At the end of 52 days' feeding, the cow was producing absolutely colorless butter. It was only when the rendered butter was viewed in the tintometer that a very slight amount of color could be detected. This very slight amount of color was due to the fact that the normal storage of pigment in the body, that in the blood serum, had not been completely exhausted, as will be shown in a subsequent paper of this series. It is probable also that the body was being drawn upon for some of its pigments, for the animal suffered somewhat from underfeeding on this ration. It is also possible that a very small amount of carotin was being supplied by the oil in the cottonseed meal and hulls. For practical purposes such a supply of pigment would of course be considered absolutely negative.

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TABLE NO. 13. EFFECT OF A LONG-CONTINUED FEEDING OF A NON-PIGMENTED RATION UPON THE COLOR OF MILK FAT. AYRSHIRE COW NO. 301.

Date of sample	Pounds alfalfa hay	Pounds cotton-seed hulls	Pounds cotton-seed meal	Pounds Corn	Color of butter fat	
					Yellow	Red
1912-13						
Nov. 5	6.5			12	9.0	1.2
6	4.5	0.5	0.5	10	8.0	1.2
7	8.0	1.0	1.0	8	9.0	1.1
8	8.0	2.0	2.0	7	8.0	1.0
9	8.0	2.0	2.0	6	7.0	1.0
10	7.0	3.0	3.0	6	6.5	1.0
11	6.0	3.0	3.0	5	6.0	1.0
12	6.0	4.0	4.0	4	5.5	0.9
13	6.0	4.0	4.0	4	5.0	0.8
14	3.0	5.0	5.0	2	5.0	0.8
15	2.0	6.0	6.0		5.0	0.9
16		10.0	6.0		4.0	0.7
17		12.0	6.0		4.5	0.8
18		12.0	7.0		4.0	0.7
19		12.0	7.0		3.5	0.7
20		12.0	8.0		3.5	0.7
21		13.0	8.0		3.5	0.7
22		14.0	8.0		3.5	0.7
23		14.0	8.0		3.2	0.7
24		16.0	8.0		3.1	0.7
25		16.0	8.0		3.0	0.7
26		16.0	8.0		2.6	0.5
27		16.0	8.0		3.2	0.7
28		16.0	8.0		2.5	0.7
29		16.0	8.0		3.0	0.7
Jan. 2		16.0	8.0		1.4	0.5
7		16.0	8.0		1.3	0.4

It will be of interest to state in connection with this experiment that it furnished the three "light colored" fats for the studies of the proportion of carotin and xanthophyll which were reported above.

Experiment 4.

The color of the butter fat of Cow No. 301 was now so low that the conditions were considered ideal for one or two additional important investigations, first a confirmation of the apparently negative effect of feeding yellow corn which was obtained in Experiments 1 and 2, and second a study of carrot feeding. In the latter study the results would be especially interesting in view of the fact that the pigment fed would be almost pure carotin. The hay used in this experiment was a very light colored timothy hay which was not quite so free from carotin and xanthophylls as the bleached alfalfa of Experiment 3, but which apparently had no effect on the color of the fat

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as the data will show. The results of the two studies are given in Table 14.

In regard to yellow corn feeding, it is seen that replacing 2 lbs. cottonseed meal and 4 lbs. white corn by 6 lbs. yellow corn had no influence upon the color of the milk fat. If yellow corn has any value as an aid in the production of yellow milk fat, it would certainly have been evident in this experiment where the milk fat was practically colorless before feeding the yellow corn. This experiment therefore gives conclusive proof of the negative value of yellow corn in the pigmentation of milk fat. The reason for this will be discussed more fully in the general discussion of the experiments.

TABLE NO. 14. EFFECT OF A RATION CONTAINING YELLOW CORN AND OF A RATION CONTAINING CARROTS UPON THE COLOR OF MILK FAT. AYRSHIRE COW NO. 301.

Date of sample		Pounds roughage(a)	Pounds carrots	Pounds cottonseed meal	Pounds corn	Color of butter fat	
						Yellow	Red
1912							
Jan.	3	14		4	4 white		
	21	12		4	4 white		
	24	12		4	4 white	1.2	0.4
	25	12		4	4 white		
	28	12		2	6 yellow		
	29	12		2	6 yellow	1.4	0.5
	30	12		2	6 yellow	1.5	0.4
	31	12		2	6 yellow	1.7	0.4
Feb.	1	12		2	6 yellow	1.7	0.4
	2	12		2	6 yellow	2.0	0.5
	3	12		2	6 yellow	1.8	0.5
	4	12		2	6 yellow	2.0	0.5
	5	12		2	6 yellow	1.8	0.3
	6	12		2	6 yellow	2.0	0.5
	7	12	6	4	4 white	2.2	0.5
	8	12	20	4	4 white	2.2	0.5
	9	12	30	4	4 white	2.3	0.5
	10	12	40	4	4 white	3.2	0.6
	11	12	40	4	4 white	4.5	0.7
	12	12	50	4	4 white	5.5	1.0
	13	12	50	4	4 white	8.0	1.0
	14	12	50	4	4 white	11.5	1.0
	15	12	30	4	4 white	16.0	1.1
	16	12	30	4	4 white	15.0	1.2
	17	12	20	4	4 white	18.0	1.3
	18		20			19.0	1.1
	19	3				15.0	1.1
	20		5			10.0	1.3
	21	3	10	1	1 white	8.0	1.2
	22	3	10	2	2 white	9.0	1.3

(a) Roughage consisted of 2 parts bleached timothy hay and 1 part cottonseed hulls.

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TABLE No. 14. (CONTINUED)

Date of sample		Pounds roughage(a)	Pounds carrots	Pounds cottonseed meal	Pounds corn	Color of butter fat	
						Yellow	Red
1913							
Feb.	23	12	20	4	4 white	11.0	1.3
	24	12	20	4	4 white	21.0	1.3
	25	12	20	4	4 white	28.8	1.2
	26	12	20	4	4 white	36.0	1.8
	27	12	20	4	4 white	27.0	1.3
	28	12	20	4	4 white	24.0	1.3
Mar.	1	12	20	4	4 white	24.0	1.3
	2	12	20	4	4 white	28.0	1.4
	3	12	20	4	4 white	26.0	1.4
	4	12	20	4	4 white	26.0	1.3
	5	12	20	4	4 white	24.0	1.4
	6	12	50	4	4 white	24.0	1.3
	7	12		4	4 white	23.0	1.3
	8	12		4	4 white	19.0	1.2
	9	12		4	4 white	19.0	1.2
	10	12		4	4 white	18.0	1.6
	11	12		4	4 white	17.0	1.1
	12	12		4	4 white	14.0	1.1
	13	14		4	4 white	11.0	1.1
	14	14		4	4 white	12.0	1.2
	15	14		4	4 white	12.0	1.2
	17	14		4	4 white	10.5	1.2
	21	14		4	4 white	7.5	1.2
	24	12		4	4 white	7.0	1.0
	30	12		4	4 white	7.5	1.3

(a) Roughage consisted of 2 parts timothy hay and 1 part cottonseed hulls.

Note: During the period from 2:18 p. m. to 2:23 a. m. the cow was badly off feed and her entire ration was withdrawn for a few days. She soon recovered, however, and was put back on the experimental ration, not feeding so many carrots, however.

In regard to the effect of feeding carrots, the data brings out several interesting points. The carrots were added to the ration on February 7, and the amount was rapidly increased to 50 lbs. per day. The cow ate them with a good deal of relish for about a week when she began to refuse part of them and finally went off feed entirely, making it necessary to withdraw her entire ration for a day or two. The effect of this carrot feeding period upon the color of the milk fat was to increase the color from 1.8 to 19 units of yellow. This increase was not nearly as great, however, as was expected considering the large amount of carrots that was fed and the length of time they had been in the ration, i. e. eleven days.

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It is clear from a study of the subsequent data that this abnormal result was due to some physiological disturbance that finally resulted in the cow going entirely off feed. As soon as the carrots were removed from the ration, the color of the fat dropped at once to 8 units of yellow. However, when the animal had recovered from her attack of indigestion, and the carrots were again added to the ration, the effect upon the color of the milk fat was perfectly normal. On the fourth day of feeding 20 lbs. of carrots the color had reached a maximum of 36 units of yellow. The color later dropped a little with an increase in fat production which is not shown in the table, but remained in the neighborhood of 28 units of yellow until the carrots were removed. The color then began to drop slowly in the normal way. After dropping to 7 units of yellow on the twenty-third day after the carrots had been removed, the experiment was stopped.

This experiment furnished the samples of butter fat whose proportion of carotin and xanthophyll were studied and reported in an earlier part of this paper, namely the fat after carrot feeding.

Experiment 5.

This was a second carrot feeding experiment using another cow, i. e. Cow No. 221, a pure bred Holstein cow. The experiment was not as successful as was hoped because of the peculiar appetite of the cow. She refused to eat more than ten pounds of the carrots per day except on two days so the experiment was discontinued. The data are given in Table 15. Notwithstanding the peculiar appetite of the cow it is interesting to note that the feeding of only 10 lbs. of carrots per day for 8 days was sufficient to bring the color of the milk fat almost back to the starting point, i. e. 26 units of yellow.

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TABLE No. 15. EFFECT OF A NON-PIGMENTED RATION AND OF A RATION CONTAINING CARROTS UPON THE COLOR OF MILK FAT. HOLSTEIN COW No. 221.

Date of sample	Pounds hay(a)	Pounds carrots	Pounds cotton-seed meal	Pounds corn	Color of butter fat	
					Yellow	Red
1912 Nov. 29	Normal herd ration containing green alfalfa hay.				26.0	1.5
Dec. 9	16		4	4	8.0	1.4
12	16		4	4	7.0	1.3
14	16		4	4	6.0	1.1
17	16	10	4	4	6.0	1.3
18	16	10	4	4	7.0	1.5
19	16	10	4	4	9.0	1.5
20	16	10	4	4	8.0	1.5
21	16	10	4	4	8.0	1.5
22	16	10	4	4	10.0	1.5
23	16	10	4	4	17.0	1.3
24	16	30	4	4	17.0	1.2
(a. m.) 25	16	20	4	4	14.0	1.5
(p. m.) 25	16	0	4	4	20.0	1.5
(a. m.) 26	16	0	4	4	18.0	1.5
(p. m.) 26	16	0	4	4	17.0	1.5
27	16	0	4	4	18.0	1.5
28	16	0	4	4	12.0	1.5
1913 Jan. 3	16	0	4	4	7.5	1.5
22	Herd ration since 1-4-13				14.0	1.2

(a) The hay was a mixture of equal parts of light colored timothy and bleached alfalfa.

Experiment No. 6

This was a feeding experiment the results of which were expected to corroborate those of Experiment 1, and show that the color of the milk fat of Jersey cows is as much dependent on the food as those of other breeds. The variation in the feed and the resulting color of the milk fat is shown in Table 16. A pure bred Jersey cow, No. 59, was used for this experiment.

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TABLE NO. 16. EFFECT OF A NON-PIGMENTED RATION UPON THE COLOR OF MILK FAT. JERSEY COW NO. 59.

Date of feeding	Pounds grain mixture(a)	Pounds corn silage	Pounds hay	Date of sample	Color of butter fat		
					Yellow	Red	Light
1911							
March 11 to April 2	11.6	0	14(b)	April 2	46.0	1.8	0.5
April 3 to April 4	11.	10	9(c)	April 14	6.0	1.5	0.2
April 15 to April 23	11.	10	8	April 23	4.0	1.4	0.2
April 24 to May 8	11.	10	4	May 9	3.0	1.5	0.2
May 10 to May 20	11	10	4	May 20	3.0	1.5	0.2
May 21 to June 14	11.5	Corn Stover 5	8(d)	June 14	47.0	1.8	0.5
June 15 to June 29	12.	5	8	June 29	26.0	2.0	0.2

(a) The grain mixture was 5 lbs. corn and 6 lbs. cottonseed meal.
(b) Green alfalfa hay from March 11 to April 2.
(c) Bleached timothy hay from April 3 to May 20.
(d) Green alfalfa hay beginning May 21.

The results of this experiment show conclusively that Jersey cows are as much dependent upon their food for the pigments of the milk fat as other breeds of cows. In addition the experiment offers excellent proof of some statements previously made in explanation of the gradual lowering of the color of the milk fat of Cow No. 57 in Experiment No. 1. It was stated there that the yellow body fat was supplementing the normal storage of pigment on account of the unpalatableness of the ration. In the present experiment we have an example of the effect of changing the ration to a non-pigmented one

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without causing the animal to draw upon any storage of pigment other than the normal one of the blood serum. The result was that the color of the milk fat dropped from 46 units of yellow to 6 units of yellow in twelve days, whereas in Experiment 1 it required thirty days to bring about a similar change of color. The very low color of the fat which was reached in Experiment 6 also indicates that only the normal storage was being drawn upon. Experiment 6 also shows that corn silage is not a source of pigment for the milk fat. The chemical changes which take place in this roughage evidently also largely destroy the carotin and xanthophylls. The chemical studies of the pigments of corn silage, which were reported above, showed this to be the case.

RELATION BETWEEN COLOR OF MILK FAT AND BREED OF COW.

The foregoing experiments have shown conclusively that dairy cows, exclusive of breed, are dependent on the carotin and xanthophylls in their feed for the pigment of their milk fat, in other words, that they cannot produce the pigment which is thus secreted. The question is at once raised as to wherein lies the so-called breed characteristic which is so much emphasized by the breeders of Guernsey and Jersey cattle? It will not be denied that a breed characteristic does exist in connection with the color of butter fat. We believe, however, that the data now to be presented will show that this breed characteristic has been overemphasized.

Since the butter fat is dependent upon the food of the cow for its color, it was necessary to compare the color of the butter fat of the different breeds under comparative feeding conditions, in order to obtain a correct estimate of the breed relation.

It would naturally be expected that the most favorable condition for studying the accuracy of the views held by the cattle breeders and others that some breeds of cows, such as the Jersey and Guernsey, are color producers while other breeds, such as the Holstein, are not color producers would be a comparison of the color of the combined fat of several cows of each breed. Table 17, which follows, gives such a comparison taken from animals in one herd. The milk and fat production of the various cows varied widely. The comparison was made during the winter months, the only source of pigment being a more or less variable quantity of green alfalfa hay in the ration, which was, however, the same for all the animals.

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TABLE NO. 17. RELATION OF BREED TO COLOR OF MILK FAT.

Breed	Color of butter fat		
	Yellow	Red	Light
Jersey	50.0	2.1	0.2
Ayrshire	38.0	1.7	0.2
Shorthorn	34.0	1.6	0.2
Holstein	31.0	1.7	0.2

The most striking fact brought out by this table is that the question of the color of the fat produced by the four breeds represented is not one of presence or absence of color, but rather a question of relative color. The fat from the Jersey cows was unquestionably the highest colored of the four samples but the fat from the Holsteins also had a very good color, although the butter would probably have been scored as "slightly low in color."

This point of relative color production is also clearly shown when comparing the fat produced by individual members of the breeds. Table 18 shows the color of the fat from two Jerseys and one Holstein cow under feeding conditions most favorable for the maximum color. These animals were producing about the same amount of butter fat, and the roughage of their ration consisted for the most part of freshly-cut soybeans, very rich in carotin and xanthophylls.

TABLE NO. 18. SHOWING RELATIVE COLOR PRODUCTION BY DIFFERENT INDIVIDUALS.

Cow No.	Breed	Feed	Color		
			Yellow	Red	Light
59	Jersey	Fresh green soybeans and grain	54.0	2.5	1.0
34	Jersey	Ditto	60.0	2.5	1.0
208	Holstein	Ditto	29.0	1.8	0.5

When comparing the color of the fat produced by individual members of the Jersey and Holstein breeds under feeding conditions favorable for only a moderate amount of color in the fat, the relative color production of the breeds very nearly approaches unity. This is

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especially true when the fat production and the actual proportion of the ration furnishing the pigments are taken into account. Such a comparison is shown in Table 19.

TABLE No. 19. RELATIVE COLOR PRODUCTION UNDER SPECIAL FEEDING CONDITIONS.

Cow No.	Breed	Pounds green alfalfa hay	Green feed (Dry matter basis) (a)	Pounds milk fat per day	Color	
					Yellow	Red
34	Jersey	10.0	42%	1.34	29.0	1.6
41	Jersey	12.0	41%	1.78	29.0	1.6
11	Jersey	8.0	40%	1.51	24.0	1.3
13	Jersey	8.0	45.5%	0.58	33.0	1.6
208	Holstein	15.0	35%	1.23	19.0	1.6
211	Holstein	12.0	28%	2.13	17.0	1.5
219	Holstein	12.0	28%	1.50	19.0	1.7

(a) Per cent of moisture free green feed in total ration.

The most interesting feature of the above table is to note that on the basis of the per cent of green dry matter in the ration, the Jersey cows produced the highest colored fat because they received the highest per cent of green dry matter. By taking into consideration also the difference in fat production, an interesting calculation can be made with the figures of average color, fat production and per cent of green dry matter in the ration, which will cause the relative color production of the two breeds to approach almost unity.

	Jerseys	Holsteins
Average per cent of green feed in ration	42.1%	30%
Average fat production	1.30 lbs.	1.6 lbs
Average color of fat (Units of yellow)	29.0	18.0

If it be assumed for the moment that there is no breed characteristic we can say that 30% green feed in the ration of the Holsteins produces 18 units of yellow in the fat for the same reason that 42% green feed in the ration of the Jerseys produces 29 units of yellow in their fat. If this is true then the following proportion would be a true one, i. e.:

$$42:29 :: 30:18$$

The product of the means is not quite equal to the product of the extremes but gives the result,

$$870 = 756$$

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If the amount of fat produced is taken into consideration and each side of this equation is multiplied by the corresponding amount of fat we have the result,

$$870 \times 1.3 = 756 \times 1.6$$
$$\text{or } 1{,}131 = 1{,}210$$
$$\text{or } 1 = 1.07, \text{ which is very near unity.}$$

The relation between the breed of the cow and the color of the fat under two different conditions of feeding is well illustrated by Tables 18 and 19. The color of the fat produced by cows No. 34 and 208 is given under both heavy and moderate pigment feeding. The data in Table 19 were obtained a number of weeks after that in Table 18. The figures show that the change from heavy to moderate pigment feeding caused the color of the milk fat of the Jersey cow to drop 50% while a similar change in the feed of the Holstein cow caused a color drop of only 35%.

The relation of the breed to the change in color produced by a change in the ration is also well illustrated in the following table No. 20.

TABLE NO. 20. COLOR PRODUCTION IN DIFFERENT BREEDS AS AFFECTED BY CHANGES IN RATION.

Cow No.	Breed	Date	Grams fat produced	Color of fat	
				Yellow	Red
213	Holstein	3-11-13	122	8.5	1.4
213	Holstein	4-10-13	135	54.0	1.8
220	Holstein	3-11-13	167	3.0	0.7
220	Holstein	4-10-13	208	22.0	1.2
303	Ayrshire	3-11-13	213	2.5	0.6
303	Ayrshire	4-10-13	263	16.0	1.1
16	Jersey	3-11-13	304	11.0	1.7
16	Jersey	4-10-13	363	64.0	2.0
57	Jersey	3-11-13	240	5.2	1.2
57	Jersey	4-10-13	263	54.0	1.7
64	Jersey	3-11-13	281	4.7	1.5
64	Jersey	4-10-13	358	47.0	1.6

The first sample for each cow in the above table represents the result of a long continued feeding of a ration almost entirely lacking in carotin and xanthophylls. The second sample represents one month's feeding of a ration rich in these pigments, the ration including a

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plentiful supply of green alfalfa hay and some fresh green grass. The data bring out two points worthy of emphasis. One of these is that it is possible to find pure bred Holstein cows entirely lacking in the so-called breed characteristic of color production. Holstein Cow No. 213 for instance produced as much color in her fat in both periods as any of the Jerseys. The low color of the milk fat of cows No. 220 and No. 303 in the second period can only be explained at present on the ground that it was due to some inherent characteristic of the animals, which for lack of a better term may be called breed characteristic. The other point brought out by the data is merely in emphasis of the results obtained in the feeding experiments showing that all breeds of cows suffer alike in regard to the color of their milk fat when the pigments carotin and xanthophylls are withdrawn from their food. At this time there is no breed characteristic.

Not only does the breed characteristic disappear when the source of the pigment is withdrawn, but it also disappears for all cows at the time of maximum color in the fat, i. e. immediately after parturition. Data was given in Table 8 showing the high color of the colostrum milk fat for cows of three breeds. There was certainly no breed characteristic evident there.

There is one other breed difference yet to be considered, which has led, probably more than anything else, to the belief that Jersey and Guernsey cows can produce yellow butter fat at any time, regardless of feed. This difference has to do primarily with the storage of pigment in the body, and its discussion belongs properly to the two subsequent papers of this series. A brief statement here in regard to it however will prevent a doubt arising in the minds of some readers, whose practical experience is apparently contrary to the experimental evidence here offered.

Stating the question in hypothetical form, it may be said that if a Jersey (or Guernsey) and a Holstein cow, both giving well-colored milk fat, the possibility of which cannot be denied in the light of the evidence which has been offered on this point, are put upon dry feed containing little or no carotin and xanthophylls, the color of the milk fat will drop much faster with the Holstein cow than with the Jersey (or Guernsey) cow, unless great care is taken to provide a ration as nourishing and palatable as the previous pigmented one. The result will be that the Jersey (or Guernsey) cow will appear to be producing colored milk fat on a non-pigmented ration. The explanation for this has already been given in connection with feeding Experiments Nos. 1 and 6, and lies in the fact that the body fat of Jersey and Guernsey cows furnishes a supplementary storage of pigments

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not usually found in other breeds. It will be shown in a subsequent paper that if the body fat which furnishes the supplementary pigments in the case of the Jersey (or Guernsey) cow is laid on with a non-pigmented ration, it will be as colorless as is often seen in the case of the body fat of Holstein cows. If this were true in the hypothetical case described above, there would have been no breed characteristic evident, for it will also be shown in a subsequent paper that the normal storage of pigment, i. e. that of the blood serum, is practically the same for all breeds of cows.

Sufficient evidence has been presented to permit a repetition of a previous statement, namely that the relation of the breed to the color of the milk fat has received more emphasis than a study of the question will warrant. The color of the milk fat is primarily dependent upon the character of the food and the fact that some breeds of cows give less color in their milk fat than other breeds will probably be found to be only an apparent one when all the factors which come into play are known.

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DISCUSSION OF RESULTS.

It was the primary object of this investigation to classify the natural yellow pigment of milk fat both as an individual and also in relation to the two well-known yellow classes of plant pigments, cartin and xanthophylls, whose general properties have often been observed to be closely related to various yellow pigments of so-called animal origin.

Basing the study upon a number of well-defined, characteristic physical and chemical properties of carotin and xanthophylls, it has been shown that the principal pigment of milk fat is a member of the fast widening group of hydrocarbon pigments, the carotin of green plants. In addition it has been shown that the milk fat carotin nearly always has associated with it one or more minor constituents whose general properties and characteristics are identical with the xanthophyll group of pigments. Two and possibly three xanthophyll constituents were found in one sample of high colored butter fat.

In addition to the establishment of a chemical relation between carotin and xanthophylls and the yellow lipochrome of milk fat, it has been possible to demonstrate a much more significant fact, namely that this lipochrome whose origin has hitherto been considered to be in the animal body is in reality merely the carotin and xanthophylls of the food, which are absorbed by the body and subsequently secreted in the milk fat. Numerous feeding experiments show that when the food is deficient in carotin and xanthophylls for a period of time, the milk fat slowly decreases in color and eventually approaches a colorless condition. The experiments also show that when foods rich in carotin and xanthophylls are given to a cow whose milk fat is deficient in lipochrome, the color of the milk fat at once increases in proportion to the amount of pigments fed. This is true regardless of whether the carotins and xanthophylls are associated with chlorophyll as in green feeds, or whether chlorophyll is completely absent and xanthophylls almost so, as in carrots.

The experiments show in addition that small amounts of carotin, such as are present in the oil of cottonseed meal have apparently no effect on the color of the butter fat. It is not clear, however, whether this is due to the smallness of the amount of carotin or to the state in which it exists in the food, i. e., dissolved in oil. There is some evidence on both sides. Mendel and Daniels[1] have recently found

1. Jour. Biol. Chem. 13, No. 1, p. 72 (1912).

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that when fifteen grams per day of Sudan III dissolved in oil was fed to a cow for three successive days, there was no indication of the dye in the milk. On the other hand, in the feeding experiments with Cow No. 301 where the bleached alfalfa hay was changed to timothy hay containing a small amount of carotin, there was also apparently no effect on the color of the milk fat.

It is especially noteworthy that all of the above feeding experiments which involved yellow corn are united in pointing to its inability to impart any color to the butter fat. This result is not so surprising, however, when viewed in the light of the character of the pigment of yellow corn as shown in the chemical studies. It was found there that the pigment is largely a xanthophyll. It may be stated that the butter fat of Cow No. 301 during the last yellow corn experiment failed to show the presence of the corn xanthophyll, when subjected to careful examination.

The feeding of carotin in the form of carrots to a cow giving as low colored milk fat as Cow No. 301 gave an excellent opportunity to study the proportion of carotin and xanthophyll in the resulting well-colored butter fat. This investigation was reported in connection with the study of the proportion of carotin and xanthophyll in butter fat under varying conditions of production. It was found that the xanthophylls were practically absent from the fat. The conclusion is that the xanthophylls must be present in the food in large excess, as in grass, before they will appear in the butter fat.

It was mentioned above, and the fact is worthy of special notice, that when the carotin and xanthophylls are withdrawn from the food the falling off in color of the butter fat is sometimes very slow. In the case of Jersey cow No. 57, it required twenty-seven days for the butter fat to drop in color from 43 to 8.5 units of yellow. Normally, it should require much less time for the color to drop this amount. For instance it required only 12 days for a similar drop to be brought about in the color of the milk fat of Jersey cow No. 59. In explanation of this difference it may be stated that upon a normal plane of nutrition, the blood serum furnishes the pigment for the milk fat. When the plane of nutrition is below normal in a lactating cow the body fat is drawn upon to aid in the production of milk fat and also for other purposes. If the body fat thus utilized has a high yellow color, as is usually the case in Jersey and Guernsey cows, the normal storage of pigment for the milk fat will be continually, at least partially, replenished. The reduction in color of the milk fat will then be much slower than normal. It was stated above and will bear repetition, that we have here an explanation of why Jersey cows apparently often produce high colored milk fat on a low pigmented ration, as during the winter months.

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The results of these experiments are of considerable practical importance. It is readily seen, for instance, that the peculiar popular conception of the enhanced value of butter on account of a high yellow color is absolutely without foundation. It is furthermore seen that the prevailing opinion among some cattle breeders that Guernsey and Jersey cows are able to synthetically produce a high colored butter fat under all conditions is also unfounded. It has been shown that all breeds of cows will produce well-colored butter fat under proper feeding conditions. The reverse has been shown to be especially true, namely that a cow, regardless of breed, cannot produce high colored butter fat under normal conditions, unless the food contains the pigments which are utilized for that purpose.

With our present knowledge, however, we would not be justified in saying that there is no breed characteristic in connection with the color of butter fat. Under apparently equal conditions Jersey and Guernsey cows usually give higher colored milk fat than Holstein or Ayrshire cows. We have been able to offer some evidence, however, showing that during a moderate pigmentation of the milk fat, this difference largely disappears when the amount of fat produced and the proportion of the ration which is the source of the pigment are taken into consideration. Further experiments would be required to ascertain whether this is true under all conditions of milk fat pigmentation or only true for moderate pigmentation. The data already at hand at least entirely justify the statement that the so-called breed characteristic has been given more emphasis than is warranted by an actual study of the facts.

The results of our experiments are furthermore of considerable physiological significance. A direct source for the lipochromes of the cow has been established, which opens the question of a similar source for all animal lipochromes. The lipochrome of milk fat has been increased or decreased with great ease by merely varying the food of the cow. Such a result throws great doubt upon any physiological significance which the lipochromes have been supposed to exert in the animals in which they have been found. Apparently it is merely a question of the inability of the animal body to throw off the excess of carotin and xanthophylls contained in its food. Experiments will reported in a later paper of this series showing that the blood serum, of the cow at least, very rapidly takes up the carotin (especially) of the food and carries it through the body in combination with an albumin of the serum.

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SUMMARY.

1. The fat of cows' milk owes its natural yellow color to the pigments carotin and xanthophylls, principally carotin, the well-known, wide-spread, yellow vegetable pigments found accompanying chlorophyll in all green plants.

2. The carotin and xanthophylls of milk fat are not synthesized in the cow's body, but are merely taken up from the food and subsequently secreted in the milk fat.

3. When food practically free from carotin and xanthophylls, such as the cow usually receives during the winter months, is given to a milk-giving cow, the immediate supply of these pigments in the organism is greatly depleted and may be entirely used up, on account of the constant drain upon the supply by the milk glands. The butter fat accordingly approaches a colorless condition in proportion to the supply of carotin and xanthophylls in the system, the length of time these pigments are kept out of the food, and also, very probably, in proportion to the amount of milk fat being produced.

4. If food rich in carotin and xanthophylls is given to a milk-giving cow whose milk fat has become practically colorless by reason of the above conditions, the organism will at once recover its lost pigments and the milk fat will increase in color in proportion to the amount of carotin and xanthophylls, especially carotin, in the food. Fresh green grass probably being the richest in carotin of all natural dairy cattle feeds, accordingly produces the highest colored butter.

5. There is some difference among different breeds of dairy cows in respect to the maximum color of the milk fat under equally favorable conditions for the production of a high color. Each breed of cows, however, will undergo the same variation in color of the milk fat which follows a withdrawal or addition of carotin and xanthophylls, especially carotin, to the food. Under some conditions, also, the apparent breed characteristic largely disappears. The popular opinion in regard to the breed characteristic has been overemphasized, and statements in regard to it should in the future be qualified with a statement of the conditions of feed, etc.

6. Under normal conditions cows of all breeds produce very high colored milk fat for a short time after parturition. The pigments of the fat at this time are identical with the normal pigments of the fat. Their increase at this time is probably due to the physiological conditions surrounding the secretion of the milk of the freshening animal.

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RESEARCH BULLETIN NO. 11.

UNIVERSITY OF MISSOURI

COLLEGE OF AGRICULTURE

AGRICULTURAL EXPERIMENT STATION

CAROTIN—THE PRINCIPAL NATURAL YELLOW PIGMENT OF MILK FAT—PART III

The Pigments of fhe Body Fat, Corpus Luteum and Skin Secretions of the Cow

COLUMBIA, MISSOURI
April, 1914

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CAROTIN—THE PRINCIPAL NATURAL YELLOW PIGMENT OF MILK FAT—Part IV. *

A. The Yellow Pigment of Blood Serum.
B. Carotin and Xanthophylls During Digestion.
C. The Pigments of Human Milk Fat.

LEROY S. PALMER and C. H. ECKLES.

A. THE YELLOW PIGMENT OF BLOOD SERUM

Very few investigations have dealt with the so-called lutein of the blood serum. Thudichum [1] was the first to mention it and classify it as a lutein. Schunck,[2] a number of years later, showed that the lutein of fowl serum was spectroscopically identical with the L. xanthophyll which he isolated from yellow flowers and green plants. Halliburton [3] also studied the lutein of the serum of the hen, but the pigment isolated by him had evidently lost its spectroscopic properties in view of Schunck's investigation. Finally, Krukenberg [4] extracted the lutein from ox serum by shaking with amyl alcohol. The extract showed two absorption bands. He used the designation lipochrome for the pigment. His work is usually mentioned in the present text books of physiological chemistry.

Recent investigations in connection with various animal luteins or lipochromes have shown that they may be classified as belonging to the widely distributed carotin or xanthophyll groups of pigments of the vegetable world. Willstätter and Escher [5] have identified the pigment of egg yolk as an isomer of the crystalline xanthophyll of green plants; and Escher [6] has shown that the principal corpus luteum pigment is identical with the carotin of plants. Extending this work

*See Research Bulletin No. 9, p. 312, for statement of Co-operation with U. S. Dept. of Agriculture.

1. Proc. Roy. Soc. 17, p. 253 (1869).
2. Proc. Roy. Soc. 72, p. 165 (1903).
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we have shown that the yellow lipochromes of the milk fat and body fat of cows are also composed principally of carotin, altho both have associated with them one or more minor xanthophyll constituents. In addition we have shown conclusively that these pigments originate from the food of the cow. They are therefore not products of animal synthesis but merely substances assimilated with the digestion products of the food and subsequently secreted in the milk fat or laid up in the body fat. The studies leading to these results are given in the two preceding papers of this series.[1]

Up to this time the experimental evidence pointing to the above stated physiological relation between the carotin and xanthophylls of plants and the pigments of butter fat and body fat has been based upon feeding experiments in which the relation between the amount of these pigments in the food and the color of the milk fat and body fat was carefully studied. It was recognized however that the evidence would not be absolutely complete until the means of transporting the food pigments to the milk fat and body fat could be established. A close study of the yellow lipochrome of the blood serum, in a manner similar to the preceding studies of the milk fat and body fat pigments, naturally seemed to offer the most ready means of establishing the physiological relation between the plant pigments and the lipochromes of milk fat and body fat.

The present investigation was therefore undertaken for the purpose of studying the yellow lipochrome of the blood serum in regard to its chemical and physiological relations to the carotin and xanthophylls of green plants and to these pigments when found in the milk fat and body fat of the cow. It was believed that this investigation would serve the twofold purpose of establishing the connecting physiological link between these plant and animal pigments and also scientifically classifying the blood serum lutein of the cow which pigment has never been the subject of close investigation.

METHODS OF IDENTIFICATION.

The methods used for identifying the pigment of the blood serum were the same as were used in the study of the milk fat and body fat pigments. They consisted in the application to the isolated pigment of the characteristic physical and chemical properties of carotin and xanthophylls. These properties were the position of the spectroscopic absorption bands, the relative solubility toward petroleum ether and 80 to 90 per cent alcohol, and the adsorption affinity toward

1. Also Jour. Biol. Chem. 17, No. 2, pp. 191, 211 (1914).

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calcium carbonate. A detailed description of these properties when applied to both the plant carotin and xanthophylls and the pigments of milk fat, body fat, corpus luteum and skin secretions of the cow was given in the two preceding bulletins [1] of this series, and need not be repeated here. The measurements of the spectroscopic absorption bands of the carotin and xanthophylls which are used for comparison were made according to an arbitrarily fixed and standard scale. It may not be out of place therefore, to repeat here a table which was given in the paper immediately preceding this one, showing these standard measurements. This table is given below as Table 1. The measurements in carbon bisulphide solution only are given.

TABLE NO. 1.—SPECTROSCOPIC STANDARDS OF CAROTIN AND XANTHOPHYLLS.

Pigment	Solvent	Measurements of absorption bands		
		Band I	Band II	Band III
Carotin	CS_2	225—242	261—278	301—319
Xanthophyll	CS_2	233—253	272—291	312—330

METHODS OF ISOLATION.

The study of the blood serum lutein required considerable preliminary study of methods of isolation. The amyl alcohol method of Krukenberg [2] was not considered suitable on account of the high boiling point of the solvent. The method used by Schunck [3] seemed to be much better suited for the work. He precipitated the proteins from the serum with alcohol, and as the proteins carried down the lutein he was able to isolate it by extracting the precipitated proteins with boiling absolute alcohol. Preliminary investigations of the blood serum lutein using Schunck's method showed, however, that it was applicable only to serum free from dissolved red blood corpuscles. When hemoglobin was present it was always carried down with the protein and some of the red color dissolved in the subsequent alcohol extract. In addition, the method did not seem to be a quantitative one, some of the lutein invariably being found in the dilute alcoholic filtrate from the precipitated proteins. These investigations showed however, that in every case both petroleum ether and carbon bisulphide almost quanti-

1. Research Bulletins Nos. 10 and 11, Missouri Agr. Exp. Sta.; also Jour. Biol. Chem. 17, pp. 191, 211 (1914).
2. Loc. cit.
3. Loc. cit.

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tatively extracted the yellow pigment from its alcoholic solution on dilution with a little water. This result at once indicated the carotin nature of the blood serum lutein, and this was confirmed by the experiments reported below.

The methods used for isolating the pigment in these studies varied somewhat in detail but were all based upon a preliminary, more or less complete dessication of the blood serum by calcium sulphate (plaster of Paris). The details are given in connection with the report of the experiments.

CHEMICAL IDENTIFICATION OF THE PIGMENT.

The following experiments were conducted to show the chemical relation of the blood serum lutein to the carotin and xanthophylls.

Experiment I.

About 20 cubic centimeters of golden yellow serum from Jersey Cow No. 8* was mixed with plaster of Paris until almost dry, dried for a few minutes on the steam bath, the mass pulverized, and shaken with successive volumes of petroleum either in an Erlenmeyer flask until no more color appeared in the petroleum ether. The extract was light yellow in color and no color was extracted from the concentrated solution by 80 per cent alcohol. The plaster of Paris mass was now shaken with successive proportions of petroleum ether containing 10 per cent absolute alcohol, until the extraction was colorless. The resulting extract had a deep yellow color containing many times as much pigment as the extract with petroleum ether alone. This solution, after concentration, was extracted with 80 per cent alcohol. Apparently no color was extracted. The petroleum ether solutions were combined, evaporated to dryness and the residue dissolved at once in carbon bisulphide giving a deep red-orange solution which showed 3 absorption bands, Band III being much fainter than the other two. The measurements of the bands are given in Table No. 2.

Experiment II.

Fifty cubic centimeters of the same serum was completely dessicated with plaster of Paris and the pulverized mass shaken with absolute alcohol and ether until no more color was extracted. The ether was distilled off and the golden-yellow alcoholic solution saponified with

*Note:—The serum in this case and in all subsequent cases was obtained by allowing the freshly drawn blood to clot in a tall cylinder or jar and the serum which pressed out on standing syphoned off into glass stoppered bottles.

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KOH. After dilution with water, the soap was extracted with ether. The ether was washed, filtered and evaporated into absolute alcohol. The alcohol was diluted to an 80 per cent solution and extracted with petroleum ether (b.p.30-50°C.) until no more color was extracted. The alcohol layer was left with quite a little color, but by far the greatest part of the color was in the petroleum ether extracts. The petroleum ether soluble pigment gave a deep red carbon bisulphide solution which showed 3 absorption bands, the third being faint. The measurements of the band are given in Table No. 2.

The 80 per cent alcohol soluble pigment showed no clear absorption bands.

Experiment III.

Fifty cubic centimeters of the same serum was mixed to a thick paste with plaster of Paris, and the pasty mass shaken thoroughly with 700 c.c. of hot 95 per cent alcohol. All the color was extracted, a second extraction with fresh alcohol being colorless. The yellow extract was concentrated to 100 c.c. An equal volume of 10 per cent alcoholic potash was added and the solution boiled on the steam bath for one hour. No aldehyde resin pigments formed. The alkaline solution was diluted with 3 volumes of distilled water and extracted with two-thirds of its volume of ether. All the color was extracted by the one extraction. After washing and filtering, the golden-yellow extract was evaporated to dryness and the residue taken up at once with petroleum ether (b.p.30-50°C.) This solution was now thoroughly shaken with 80 per cent alcohol until no more color was extracted. Fresh petroleum ether extracted a little color from the alcoholic extract, which was not re-extracted by fresh 80 per cent alcohol. The blood serum lutein was now divided into a major and minor pigment, the major being insoluble in 80 per cent alcohol in the presence of petroleum ether and the minor being insoluble in petroleum ether in the presence of 80 per cent alcohol.

The petroleum ether soluble pigment had a blood-red color in CS_2 solution and showed 3 absorption bands, the measurements of which are given in Table No. 2.

Analyzed chromotographically it passed through $CaCO_3$ unadsorbed as a beautiful rose-colored area, leaving no adsorbed zones.

The 80 per cent alcohol soluble pigment was transferred to ether by diluting its alcoholic-ether solution with much water, and from the ether to carbon bisulphide after evaporation of the former. In carbon bisulphide it gave an orange-yellow solution showing two absorption bands in the 25 m.m. cell. The measurements of the bands are given in Table No. 2.

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Chromotographic analysis showed the presence of only one pigment which very slowly passed through the $CaCO_3$ as a yellow zone.

Experiment IV.

Serum from Jersey Cow No. 2, to the amount of 275 cubic centimeters, was dessicated with a little more than the calculated amount of plaster of Paris necessary to take up the water, and after setting over night the hard mass was pulverized in a mortar. The powder was moistened with 95 per cent alcohol and shaken with petroleum ether (b.p.30-50°C.) until the petroleum ether extracted no more color, and then with ether until that extract was colorless.[1] The petroleum ether extract was concentrated to 50 c.c. and the solution added to the alcohol-free ether-alcohol extract, which had been concentrated to about 150 c.c. An equal volume of 4 per cent alcoholic potash solution was now added to the combined ethereal solutions, the ethers evaporated off and the alcoholic solution boiled on the steam bath for a few minutes. The pigment was then extracted from the alkaline alcoholic solution in the usual way and when in alcoholic solution was analyzed with respect to petroleum ether and eighty to ninety per cent alcohol. Two pigments were thus obtained with proportions of perhaps 95 and 5 per cent of the total.

The petroleum ether soluble pigment gave a red colored residue which dissolved instantly in carbon bisulphide with a blood-red color, and showed the most beautiful absorption bands yet seen for this pigment. Three bands were visible, the third band being considerably fainter than the other two bands but clear and distinct. The measurements are given in Table No. 2.

1. The mechanism of this method of obtaining the blood serum pigment is so interesting, its advantages so striking and its results so satisfactory, that it is worthy of some discussion.

It appears that the addition of just sufficient alcohol (either absolute or 95 per cent) to thoroughly moisten the dessicated serum liberates the main lutein pigment in such a way that when the moistened mass is shaken with petroleum ether the result is the same as if an 80 per cent to 90 per cent alcoholic solution of the isolated pigment is shaken with petroleum ether. There is the additional advantage however, that the $CaSO_4$ prevents the formation of emulsions and holds the alcoholic solution so firmly fixed in the paste that the petroleum ether can be poured away and the use of a separatory funnel be entirely dispensed with. When all the pigment more soluble in petroleum ether than in 80 per cent alcohol is thus extracted, any pigment which remains can be readily extracted with ether which mixes readily with the dilute alcohol. The pigment thus extracted can be readily freed from alcohol by shaking with water leaving the last as well as the first pigment extracted, in low boiling point solvents, an additional decided advantage in view of the ease with which they are oxidized. It should be added however that the method for extracting the pigment more soluble in alcohol than in petroleum ether does not apply well for serum containing much haemoglobin for in this as well as all other alcohol methods the red pigment is somewhat soluble in the dilute alcohol.

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Analyzed by means of a chromotogramm the solution showed only a wide, quickly filtering, unadsorbed, rose-colored zone.

The 80 per cent alcohol soluble pigment was transferred to ether and then to carbon bisulphide, giving in the latter an orange-yellow solution showing one band and much end-absorption. In the chromotogramm it showed 2 zones close together, an upper orange zone and a lower canary yellow zone. The carbon bisulphide solution of the orange zone showed 2 absorption bands and end-absorption. The measurements are given in Table No. 2.

TABLE No. 2.—SPECTROSCOPIC ABSORPTION BANDS OF BLOOD SERUM CAROTIN AND XANTHOPHYLLS.

Experiment No.	Solvent	Absorption bands	
		Carotin	Xanthophylls
1	CS_2	I. 225—242 II. 263—286 III. 305—322	
2	CS_2	I. 223—243 II. 262—285 III. 302—325	
3	CS_2	I. 225—242 II. 263—286 III. 305—325	I. 232—254 II. 273—295 III.
4	CS_2	I. 223—242 II. 262—286 III. 300—320	I. 232—252 II. 270—292 III. 310—

DISCUSSION OF EXPERIMENTS.

It must be concluded from the above experiments that the principal lipochrome of the blood serum of the cow is identical with that of the milk fat, body fat, and corpus luteum, and as in the case of these pigments, with the carotin of green plants.

It appears also from the above investigations that a small portion of the blood lutein pigment is composed of xanthophylls. It was found to be much more difficult to show their presence in the blood serum than in the body fat or butter fat. The reason for this is not perfectly clear but a close study of the investigations throws some light on the question. It will be noticed that it required complete extraction of

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a comparatively large amount of serum with ether and subsequent saponification of the fat thus extracted to really demonstrate the presence of xanthophylls. It is a well-known physiological fact that the proportion of fat in blood serum is comparatively small.* When coupled with the above observations, this seems to indicate a relation between the xanthophylls and the fat carried by the blood. Some observations which will be reported later, in connection with the fate of the carotin and xanthophylls during digestion, will furnish more evidence in this same direction.

PHYSIOLOGICAL RELATION BETWEEN CAROTIN OF BLOOD SERUM AND FOOD OF COW.

After establishing the chemical relation between the principal blood serum lipochrome and the carotin of the food, it became important to establish a similar relation from a physiological standpoint. Very fortunately this was recognized previous to conducting some of the important feeding experiments which showed the relation between the color of milk fat and the food of the cow, and which were reported in Research Bulletin No. 10,[1] Missouri Agr. Exp. Sta., the second bulletin in this series. It was accordingly arranged to study the variation in the amount of carotin and xanthophylls in the blood serum during portions of these feeding experiments. In this way it could be determined what relation exists between the amount of carotin and xanthophylls in the blood serum and the amount of these pigments in the milk fat, as well as the relation between the amount of carotin and xanthophylls in the serum and amount of these pigments in the food. Such a study required the devising of some method of analysis whereby the color of the various blood serums could be compared with each other and also with the color of the butter fat. The following method was adopted.

In the case of live cows whose blood was to be tested, a trocar was inserted in the jugular vein and 200 to 250 c. cm. of blood drawn off into a glass cylinder. As soon as the blood had clotted and sufficient serum had pressed out, two 10 cc. portions were pipetted off and carefully dessicated with an excess of plaster of Paris. The powdered mass was moistened with absolute alcohol, and the color extracted immediately by shaking with the selected solvent until colorless. For one sample the solvent was ether and for the other sample the solvent was petroleum ether. In all the studies the petroleum

*_Note_:—Hammerstein gives .1 to .7%.

1. Also Jour. Biol. Chem. 17, p. 191 (1914).

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ether extract proved to be the easiest to handle as it was practically free from water. The extract in each case was carefully evaporated to a volume of 1 to 2 cubic centimeters and then made up to 12½ cubic centimeters with absolute alcohol, this volume being just sufficient to fill the one-inch cell of the Lovibond Tintometer. The solutions were analyzed at once in the Tintometer and their color readings recorded. Duplicate determinations were thus obtained. This was considered necessary since the method was not free from error due to possible bleaching of the extracted pigments. The entire procedure was carried out as quickly as possible. The results of the duplicate determinations were averaged.

The first series of observations of the color of the milk fat and blood serum corresponding to various pigmented rations was made with Ayrshire cow No. 301. These feeding experiments and the resulting variation in the color of the milk fat were shown in detail in Tables 12, 13 and 14 of the preceding bulletin dealing with the milk fat pigment. The relation between the character of the ration, and the color of the milk fat and blood serum at stated intervals during the feeding experiments is shown in Table No. 3, below.

TABLE No. 3.—THE RELATION OF THE CHARACTER OF THE RATION TO THE COLOR OF THE MILK FAT AND BLOOD SERUM.
AYRSHIRE COW No. 301.

Date of sample.	Feed of cow	Butterfat		Serum	
		Yellow	Red	Yellow	Red
1913					
Jan. 7	Cottonseed meal and cottonseed hulls only.....	1.3	0.4	3.3	0.5
Jan. 24	Cottonseed hulls, timothy hay and white corn.....	1.2	0.4	2.6	1.1
Feb. 7	Timothy hay, cottonseed hulls, cottonseed meal and yellow corn.....	2.0	0.5	4.9	1.2
Mar. 1	Timothy hay, cottonseed hulls, cottonseed meal, yellow corn, and 20 lbs. carrots per day.....	24.0	1.3	54.0	1.8
Mar. 6	Timothy hay, cottonseed hulls, cottonseed meal, yellow corn, and 20 lbs. carrots per day.....	24.0	1.4	47.0	1.5
Mar. 27	Timothy hay, cottonseed hulls, cottonseed meal, and yellow corn.....	7.0	1.0	26.0	0.7

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The above table shows in a very striking manner that the amount of carotin in the blood serum of the lactating cow, as well as the amount of carotin and xanthophylls in the milk fat, is dependent upon the ration. The figures in the table also show very clearly why the butter fat in the first two cases was not absolutely colorless. These two samples were taken at the end of a long-continued feeding of a ration almost entirely lacking in carotin and xanthophylls, which was planned for the purpose of eliminating as far as possible the color from the milk fat. It is evident that this was not accomplished in the strictest sense of the word because the blood serum in both cases still contained a small amount of carotin.

The results of these studies were so striking that it was considered advisable to confirm them if possible with other animals. We fortunately had at hand, 6 cows of 3 different breeds, all pure bred animals, which were in ideal condition for such an experiment. They had all just completed an experiment in which their feed for 12 to 14 weeks had been essentially a non-pigmented one, being made up of cottonseed meal, corn stover and very light-colored timothy hay. A night and morning milking of each cow was combined and after determining the percentage of fat in the combined sample [1] the milk was separated, the cream churned, and the color of the rendered butter fat observed in the Tintometer. The same day, samples of blood were drawn from each animal and the color of the serum determined by the method described above. The feed of the cows was now changed so that it was largely made up of alfalfa hay, rich in carotin and xanthophylls, and later a little fresh green pasture grass. Thirty days after the cows had been on this feed the first experiment was repeated and the color of the butter fat and blood serum again observed. The results of this experiment are given in Table No. 4.

1. The weight of the combined sample was also recorded.

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TABLE NO. 4.—RELATION BETWEEN CHARACTER OF RATION AND AMOUNT OF PIGMENT IN MILK FAT AND BLOOD SERUM.

Cow No.	Breed	Date	Grams fat.	Butterfat		Serum	
				Yellow	Red	Yellow	Red
213	Holstein	3–11–13	122	8.5	1.4	6.0	0.7
213	Holstein	4–10–13	135	54.0	1.8	48.0	1.1
220	Holstein	3–11–13	167	3.0	0.7	7.0	0.8
220	Holstein	4–10–13	208	22.0	1.2	41.0	1.0
303	Ayrshire	3–11–13	213	2.5	0.6	11.0	0.9
303	Ayrshire	4–10–13	263	16.0	1.1	40.0	1.0
16	Jersey	3–11–13	304	11.0	1.7	10.0	0.9
16	Jersey	4–10–13	363	64.0	2.0	45.0	1.1
57	Jersey	3–11–13	240	5.2	1.2	13.0	1.1
57	Jersey	4–10–13	263	54.0	1.7	57.0	1.8
64	Jersey	3–11–13	281	4.7	1.5	7.5	0.7
64	Jersey	4–10–13	350	47.0	1.6	45.0	1.0

The results of this experiment are as striking and conclusive as in the experiment with Cow No. 301, and show that there is a direct relation between the amount of carotin in the food and the amount of lutein in the blood serum, just as there is a direct relation between the presence of an excess of carotin in the food and the production of a high-colored butter fat. It is necessarily true also that there is a direct relation between the color of the butter fat and the amount of lutein in the blood serum. A small amount of lutein in the blood serum will always mean a light-colored butter fat. It does not appear to be necessarily true, however, that a high-colored serum will be accompanied by a high-colored butter fat. The only conclusion in this connection that can be drawn from the data of Tables 3 and 4 is that an increase in the color of the blood is accompanied by an increase in the color of the fat. The actual color of the fat under these conditions is apparently dependent upon a number of conditions which are not explained by this data. The amount of fat being produced and the breed of the animal are both factors which probably influence the color of the fat. There is certainly a wider difference

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between the color readings of the butter fats than between the color readings of the blood serum extracts during the second part of the experiment whose data are given in Table No. 4.

It is possible that this difference may be explained on the ground that the albumin content of the milk is in some way closely related to the color of the milk fat. At any rate the data given in the following table admit of such interpretation. The conditions of the animal preceding the data were as follows: Cow No. 301 had been subjected to severe underfeeding, i. e., she received only about 70 per cent of the food required to produce her milk and maintain her body weight. The food she received during this time was composed of about 5 pounds of a mixture of corn, bran and linseed meal, and about 7 pounds of alfalfa hay, a ration moderately rich in carotin. Her ration was then changed to one practically free from carotin, consisting of white corn, cottonseed meal and bleached alfalfa hay. The cow was brought back to a normal plane of nutrition on this ration. The immediate effect on the composition of the milk and the color of the milk fat is shown in Table 5. The subsequent effect upon the color of the milk fat is given in Table No. 15 of the preceding Bulletin of this series which dealt with the milk fat pigment.

TABLE NO. 5.—A POSSIBLE RELATION BETWEEN THE ALBUMIN OF MILK AND THE COLOR OF THE MILK FAT.
AYRSHIRE COW NO. 301.

Date 1912	Hay Lbs.	Grain Lbs.	Milk fat per day Grams	Total protein Grams	Casein Grams	Albumin Grams (c)	Color of fat	
							Yellow	Red
9–23–24	7.2(a)	4.9	254	209	138	37	15	1.8
9–25–26	8.1(b)	4.9	249	203	134	36	15	1.8
9–27–28	11.0	6.0	262	222	138	47	19	1.8
9–29–30	14.0	7.0	272	230	135	57	21	1.7
10–1–2	14.5	7.5	267	237	132	67	28	1.8
10–3–4	9.8	8.0	276	220	139	46	20	1.7

(a) Alfalfa hay, rich in carotin.
(b) Bleached alfalfa hay, free from carotin.
(c) Calculated.

The data in Table No. 5 shows that although the conditions of feed were such that a decline in color would be expected, the reverse was found. The point to be emphasized is that the sharp increase in the color of the milk fat was coincident with an increase in the

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albumin content of the milk. The result points to the probability of a relation between the higher color and the increase in albumin. Additional evidence pointing to the same relation is the presence of extremely high color along with a very abnormal amount of albumin in colostrum milk, as pointed out later in this paper.

An attempt was made to determine if any definite relation exists between the albumin and color as found in the milk of various individuals. The milk of 12 cows representing the Jersey, Holstein and Ayrshire breeds was used. The feed received was pasture grass and some grain. The color of the milk fat and the percentage of albumin were determined for each animal. The results of the study are given in Table No. 6.

TABLE NO. 6.—RELATION BETWEEN THE ALBUMIN CONTENT OF MILK AND THE COLOR OF MILK FAT, UNDER NORMAL CONDITIONS.

Breed	Cow No.	Pounds milk per day	Grams protein per day	Grams albumin per day	Grams fat per day	Color of fat.	
						Yellow	Red
J.	14	18.4	311.0	24.45	459.0	64.0	2.7
J.	64	12.9	254.0	22.65	380.2	64.0	2.8
J.	57	10.8	202.0	22.02	267.0	64.0	3.0
A.	303	20.2	310.0	33.20	363.5	50.0	1.7
H.	221	15.1	270.0	27.05	257.0	43.0	1.8
H.	222	14.5	233.8	20.12	202.5	33.0	1.3
J.	16	20.8	339.0	35.75	414.0	64.0	3.1
J.	317	13.0	234.5	41.00	259.0	80.0	3.5
H.	225	13.3	200.0	22.35	150.9	47.0	1.8
H.	220	16.3	525.0	35.17	192.5	24.0	1.7
A.	301	18.7	282.5	39.00	273.0	54.0	2.0
H.	213	11.4	161.5	19.05	144.2	64.0	2.7

"J." stands for Jersey, "H." for Holstein, "A." for Ayrshire.

There appears to have been no relation between the albumin of the milk and the color of the milk fat of these animals. It is not considered however, that these results are conclusive either as proving or disproving the supposition that such a relation exists. Our knowledge of the subject is too limited at present to enable us to control all the factors that enter into the question.

THE TRANSPORTATION OF CAROTIN AND XANTHOPHYLLS BY THE BLOOD SERUM.

When it had been shown conclusively that the lutein of the blood serum of the cow is composed of the carotin and xanthophyll pigments of the food, taken up along the digestive tract and transmitted by means of the blood to the fat synthesizing cells of the milk glands

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and body tissue, it became a matter of considerable importance to ascertain how the blood carries these pigments through the body. It has already been shown that there is a strong possibility that the minor constituent of the pigment, i.e., the xanthophylls, are carried in the serum dissolved in the fat. When considering such physiological phenomena as the great volume of blood in circulation in an animal as large as the cow and the rapidity with which it circulates, it would seem very probable that even the very small percentage of fat in the blood is sufficient to account for all the pigment, both carotin and xanthophylls, which is presented to the milk glands and body cells. On the other hand, when one considers the very large proportion of carotin which is present in any given quantity of blood serum of a cow receiving a ration rich in carotin, it must be concluded that the fat plays little if any part in the transportation of this pigment. The studies that are to be presented will, therefore, consider only the carotin of the blood serum, since it is this pigment that comprises by far the greatest proportion of the lutein of the serum.

It might be considered that the carotin is carried by the serum merely in simple solution. In fact, Thudichum [1] stated that the lutein of the blood is in solution in the serum. This seems to be very probable especially in view of the fact that Krukenberg [2] found that it could be extracted from the serum by means of amyl alcohol.

Many facts can be presented, however, that go to show that the carotin does not exist in the serum in simple solution. In the first place neither carotin from plants nor the carotin of the serum itself are, when isolated, taken up to any extent when treated with the pure blood serum. Blood serum almost free from carotin from natural causes, showed no indication of having taken up the carotin in either case when poured over the pure amorphus pigments; and the serum itself showed no increase in the amount of color that could be extracted by petroleum ether after dessication with plaster of Paris and moistening with alcohol.

In addition to the above the following observations were made: [3]

1. Five c.c. portions were shaken vigorously with equal volumes of petroleum ether, ether, CS_2 and amyl alcohol respectively. All extracts were colorless except in the case of the amyl alcohol which was golden-yellow, and showed the carotin absorption bands both

1. Loc. cit.
2. Loc. cit.
3. Except where stated most of work about to be reported was done with a golden-yellow, high-colored serum from Ayrshire cow No. 301. The serum was obtained from this cow by drawing 250 c.c. of blood from the jugular vein and allowing it to clot and the serum to press out. The serum was free from red corpuscles.

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in carbon bisulphide and alcohol, the solution in the first solvent showing three distinct bands. This shows that the lipochrome which Krukenberg extracted from ox serum was the pigment which we have identified as carotin. On addition of alcohol to the other mixtures the solvents in each case completely extracted the pigment on shaking.

2. Five c.c. portions were dessicated with plaster of Paris and shaken with ether, petroleum ether, and carbon bisulphide, respectively. A mere trace of color was extracted in each case. When a little absolute alcohol was added however, in all cases the solvents became well colored.

3. Five c.c. of serum was diluted with twenty volumes of water, without causing any precipitation of the pigment.

4. Twenty-five c.c. of serum was treated with successive portions of saturated $(NH_4)_2\ SO_4$ solution to the following per cent saturations: 28-35, 36-40, 45-46, and finally to one-half saturation. The fractionally precipitated globulins were in every case practically free from carotin, and the half saturated globulin free serum was golden-yellow. The color was entirely precipitated from a portion of this solution on complete saturation with $(NH_4)_2\ SO_4$. The remainder was acidified with a few drops of 1½ per cent acetic acid and heated to about 80°C. The coagulated albumins carried down only a small part of the color. The entire pigment was precipitated from the filtrate, however, on complete saturation with $(NH_4)_2\ SO_4$ in substance, the light precipitate which came down being deep yellow in color. This deep yellow precipitate was readily soluble in water giving a clear yellow aqueous solution from which neither ether nor petroleum ether would extract any color until the protein in the solution had first been coagulated with alcohol.

5. Five c.c. of serum was diluted to 25 c.c. with distilled water and the solution saturated with $Mg\ SO_4$ in substance. The globulins were filtered off. The filtrate was golden-yellow. Acetic acid was added to a concentration of 1 per cent. The precipitated albumins were bright yellow, leaving the solution colorless. Petroleum ether and carbon bisulphide extracted a slight amount of color from this precipitate on long contact. After the addition of a little alcohol, however, both solvents readily extracted the color.

6. One hundred c.c. of serum from Jersey Cow No. 25 was diluted with several volumes of water, a pinch of NaCl and a few drops of glacial acetic acid added and the solution heated quickly to a temperature just below the boiling point. The coagulum which formed contained a very little pigment but the filtrate was golden-yellow. No color could be extracted from the filtrate by carbon bisulphide, or by

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ether, even after making strongly alkaline with potassium hydroxide. Amyl alcohol extracted the pigment.

7. Experiment 6 repeated on 200 c.c. of the same serum gave the same result. The entire pigment in the golden-yellow filtrate was coagulated by boiling. The coagulum was not soluble in water.

8. Fifty c.c. of serum (Cow No. 2) was diluted to 350 c.c. with water, a pinch of salt added and the solution heated on the steam bath, with stirring until cloudiness appeared. On adding a few drops of glacial acetic acid, a sharp coagulation took place. On filtering, the filtrate was bright yellow in color. On saturation of the filtrate with $(NH_4)_2\ SO_4$ in substance, a comparatively small amount of deep yellow precipitate was thrown down leaving a colorless supernatant solution. The yellow precipitate, which was contaminated with a little $(NH_4)_2\ SO_4$, was readily soluble in water, giving a perfectly clear yellow solution from which the yellow color was again entirely thrown down on saturation with $(NH_4)_2\ SO_4$ in substance, or on the addition of mercuric nitrate. The latter precipitate when still moist would not give up its color to petroleum ether until first moistened with absolute alcohol. The bright yellow pigment now found in the petroleum ether gave a red-orange CS_2 solution which showed the three carotin absorption bands.

9. Two 350 c.c. portions of serum (Jersey Cow No. 2) were treated respectively as follows:

Portion A was treated with $(NH_4)_2\ SO_4$ in substance to one half saturation, according to the formula

$$X = \frac{VC_2}{18.158 - .54\ C_2}$$

Where V = original volume of protein solution.
C = desired saturation as grams in 10 c.c.
X = grams to be added to give the required saturation.

The globulins which precipitated carried down some of the pigment, but on dissolving them in 150 c.c. of warm water containing some $(NH_4)_2\ SO_4$, and adding $(NH_4)_2\ SO_4$ to half saturation, they were thrown down practically colorless. The yellow filtrate from this precipitation was added to the other globulin-free filtrate and the combined solutions diluted to 1500 c.c. with distilled water. This solution was now raised to a temperature of 75°C in a water bath. 15 c.c. of 1½ per cent acetic acid added and the temperature raised to 80°C, when a sharp coagulation occurred. The solution was filtered, giving a

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perfectly clear bright yellow filtrate. It was saturated with $(NH_4)_2 SO_4$ in substance, throwing down a small amount of deep yellow precipitate. The precipitate was filtered off on a large (11-inch) Büchner funnel, using suction, so that the layer of yellow protein would be as free as possible from occluded $(NH_4)_2 SO_4$. The golden-yellow precipitate was sucked as dry as possible on the funnel and the sticky mass covering the paper in very thin layer dissolved in warm water, in which it was readily soluble, and the clear yellow solution set aside.

Portion B was diluted with an equal volume of distilled water, a little sodium chloride added, and the solution raised to a temperature of 75°C. in a water bath. Acetic acid was now added carefully until a heavy definite coagulation took place. The coagulated proteins carried down some of the pigment but by far the greatest part was in the clear yellow filtrate. This filtrate was saturated with $(NH_4)_2 SO_4$ in substance and the precipitated pigmented protein filtered off in the same way as in the case of portion A. After being made comparatively dry by suction, the deep yellow residue was readily soluble in a small amount of cold distilled water.

The two similar solutions from A and B were now combined and filtered on a small Büchner funnel through several layers of fine paper to free it from dirt and other foreign matter introduced by the $(NH_4)_2 SO_4$. The golden-brownish-yellow filtrate of about 250 c.c. volume had a faint cloudiness when viewed by transmitted light and contained some $(NH_4)_2 SO_4$. That the pigment of this solution was carotin was shown by the fact that when an equal volume of alcohol was added to 15 c.c. of the solution and the mixture was shaken with petroleum ether, the petroleum ether rose to the top as a golden-yellow solution, leaving the lower cloudy alcoholic layer colorless. The pigment in the petroleum ether layer gave a red-orange carbon bisulphide solution, and in this solvent showed the usual carotin absorption bands.

The aqueous solution was now dialysed in a parchment bag against running water for eight days. At the end of this time the solution was still giving a precipitate with barium chloride indicating that the solution was not free from $(NH_4)_2 SO_4$. No protein crystallization had occurred, but decomposition had begun, for the solution was cloudy, and showed a very fine coagulation. This coagulum was filtered off. It had a dirty brown color and when almost dry was quite sticky. It was not soluble in water, but both in the dry state and in suspension in water it gave up a golden-yellow color to ether, leaving the precipitate dirty white in color. The extracted pigment showed the three absorption bands of carotin in carbon bisulphide solution.

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In the solid state the pigment was insoluble in absolute alcohol but readily soluble in petroleum ether—absolute alcohol, from which the petroleum ether readily extracted it on dilution with a little water. The solid pigment was also very difficultly soluble in alcoholic potash. After saponification for one half hour and extraction by ether, the pigment, in the solid state, was fused with a little solid sodium hydroxide and potassium nitrate in a porcelain crucible. The flux was dissolved in hot water and the solution evaporated to dryness on the steam bath in the presence of an excess of c. p. HNO_3. As much as possible of the residue was dissolved in hot water containing some c. p. HNO_3, the solution filtered and an equal volume of pure molybdate solution added to the 100 c.c. of filtrate. On digestion at 60°C, for several hours there was a distinct yellow precipitate of ammonium phosphomolybdate.

Returning to the slightly cloudy but yellow aqueous filtrate from the dialysed solution, we found that the color could be entirely thrown down; (1) by acid lead acetate as a light yellow precipitate which bleached almost entirely in 12 hours, but from which petroleum ether extracted a faint yellow color after soaking in alcohol for about one hour; (2) by nitric acid mercuric nitrate solution as a bright yellow precipitate which was very stable and gave up its color to petroleum ether only after soaking in alcohol;[1] (3) by neutral ten per cent solution of $AgNO_3$ as a deep yellow precipitate which was stable although darkening badly as the $AgNO_3$ oxidized in the light, but readily giving up its color to petroleum ether on addition of alcohol to the precipitate, the pigment thus extracted showing the carotin bands in CS_2 solution; (4) on saturation with $(NH_4)_2\ SO_4$ in substance as a deep yellow precipitate which was not soluble in water but when suspended in water gave up its color to petroleum ether only after the addition of absolute alcohol; (5) on addition of an excess of alcohol as a yellow precipitate which when dry gave up no color to petroleum ether alone, but to alcoholic petroleum ether gave up a yellow pigment which was quantitatively found in the petroleum ether on separation of the alcohol with a little water; (6) on heating the neutral solution to boiling as a yellow coagulum insoluble in water and giving up no color to hot alcohol or petroleum ether.

In addition to the above observations the following may be mentioned. In working with a large number of samples of blood serum

1. The pigment thus extracted showed the three carotin absorption bands in carbon bisulphide solution; in alcoholic solution it gave a pronounced precipitate of digitonin-cholesteride on addition of hot one per cent digitonin solution in ninety per cent alcohol.

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it was often noticed that when the serum had stood for some time in a closed bottle in contact with a little supernatant air, an orange-yellow scum always came to the top. This was found to be a water insoluble protein which would not give up any color to petroleum ether when its aqueous suspension was shaken with that solvent, but when an equal volume of absolute alcohol was added and the shaking with petroleum ether repeated, the latter solvent rose to the top as a beautiful yellow layer. The pigment thus extracted gave a red-orange carbon bisulphide solution showing the three carotin absorption bands.

In order to show more conclusively the character of the protein with which the serum carotin is evidently combined, the coagulation temperature of the protein was determined. For this purpose 150 c.c. of serum was diluted with an equal volume of a saturated solution of ammonium sulphate. After filtering off the precipitated globulins, portions of the globulin-free filtrate were submitted to fractional coagulation. It was found that on carefully elevating the temperature to 80° C. and holding it at that temperature for a short time, the filtrate from the coagulated albumins still yielded a large amount of carotin on addition of alcohol and shaking with petroleum ether. On the other hand the coagulated albumins yielded a comparatively small amount of carotin. A similar result was obtained at temperatures of 81°, 82°, 83°, 84°, 85°, and 85.5° C., although the amount of carotin in the filtrate rapidly decreased with the increase in coagulation temperature. At 86° C., however, the pigmented protein had completely coagulated, and the filtrate yielded no carotin on treatment with alcohol and petroleum ether. The coagulation temperature limits of the pigment carrying protein therefore lie between 80° and 86° C., when the protein is in half saturated ammonium sulphate solution. There is no marked coagulation at the lower temperature, but it is completely coagulated at the upper temperature.

The coagulation temperature of the protein which carries the carotin in the blood was studied further with an aqueous solution of the protein obtained in a manner similar to the one used in obtaining the protein for the study previously reported. Briefly, an equal volume of saturated $(NH_4)_2\ SO_4$ solution was added to 200 cubic centimeters of blood, rich in carotin, from Holstein Cow No. 221. The globulins were filtered off and the golden-yellow filtrate heated carefully in a water bath to a temperature of 79° C. The coagulated proteins were filtered off. The yellow filtrate was saturated with $(NH_4)_2\ SO_4$ in substance and let stand several hours. The golden-yellow precipitate was filtered off on a Büchner funnel. After allowing to suck

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quite dry, all crystals of $(NH_4)_2\ SO_4$ were removed with a spatula and the protein dissolved in about 75 cubic centimeters of water, in which it was readily soluble. This deep yellow solution was neutral in reaction. It contained a small amount of $(NH_4)_2\ SO_4$, the amount of which was determined quantitatively. $(NH_4)_2\ SO_4$ was added to a portion of this solution to bring the concentration up to a normal solution. An equal volume of saturated $(NH_4)_2\ SO_4$ solution was added to the remainder, giving a solution between 3 and 4 normal and one similar to the one whose coagulation temperature was observed above. The coagulation temperature of the pigmented protein of these two solutions was carefully studied. Both solutions were found to contain a small amount of colorless protein which coagulated between 65° and 75° C. This was filtered off, the filtrate retaining its original yellow color. This filtrate was then studied further.

The 3-4 normal or one half saturated $(NH_4)_2\ SO_4$ solution acted in a manner identical with the solution whose study is recorded above. The first opalescence appeared between 79° and 80° C., and complete coagulation did not take place until the temperature was raised to 86° C.

It was not found possible to cause a clear coagulation of the pigmented protein in the neutral normal solution of $(NH_4)_2\ SO_4$ even when the temperature was raised to 90° C. Opalescence began, however, between 82° and 82.5° C. Coagulation was readily obtained when the solution was heated to 89° C. in the presence of a very little HCl. (3 drops of a ½ normal HCl solution were added to 10 c. c. of solution.)

The large amount of evidence which has now been submitted in regard to the transportation of the carotin in the blood serum will justify but one conclusion, namely that the carotin exists in the blood in conjugation with one of the proteins. The evidence will also justify the conclusion that the protein with which the carotin is combined is an albumin.

Summarizing the evidence, we have shown that the carotin carrying protein is precipitated from its solution in the serum or from its aqueous solution, on complete saturation only with ammonium sulphate, or by saturation with magnesium sulphate only in one per cent acetic acid solution, or by heating its half saturated ammonium sulphate solution to 86° C.; the protein may also be coagulated by alcohol, or by boilding its solution in the presence of acetic acid. As in all salting out methods for the precipitation of proteins, the pigmented protein is readily soluble in water after being thrown down by ammonium

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sulphate, but is no longer soluble in water after being coagulated by heat or alcohol.[1] The protein is not coagulated by saturation of its solution with sodium chloride.

Not very much can be said in regard to the character of the combination of the carotin with the albumin The combination is evidently a firm one, and is broken down only in the presence of alcohol so that the pigment can be extracted by ether or petroleum ether. The union is also broken down by dialysis, or at least rendered less firm, but is not broken down when the protein is precipitated as a lead, silver or mercury salt. It is interesting to notice that cholesterol and a phosphorus-containing body (probably lecithin) are mixed up in some way in the combination of albumin and carotin, the liberated carotin from the dialysed pigmented albumin yielding both cholesterol and phosphorus. We propose the name caroto-albumin or luteo-albumin for the chromo-protein which transmits the carotin from the food to the milk glands and fat synthesizing body cells of the cow.

The finding of this highly unsaturated hydrocarbon carotin pigment in combination with one of the albumins of the blood, probably similar to the combination of the haematin in the haemoglobin of the red blood corpuscles, at once raises some important questions as to a possible physiological significance which might be attached to the presence of the pigment. One can only suggest that like the haemoglobin the luteo-albumin may be of importance in connection with the oxygen supply of the body. This is not probable. The ease with which the carotin is increased and decreased in the blood serum as shown by the feeding experiments, seems to preclude the possibility of the carotin being absolutely essential to the life of the cow.

A STUDY OF THE HIGH COLOR OF COLOSTRUM MILK FAT.

Considerable data was given in the preceding Bulletin of this series, which showed that colostrum milk fat from all breeds of cows is characterized by a very high content of carotin. In view of the results obtained in the study of the pigment of the blood serum, it seemed very probable that this interesting phenomenon was due to a great accumulation of the carotin in the blood serum just previous to parturition. In order to obtain some definite experimental evidence in support of this supposition, blood was drawn from the jugular vein of a pure bred Jersey cow (No. 23), when she was dry and three days previous to parturition. The amount of color in 10 c.c. of the blood

1. After standing a short time under the alcohol.

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was determined in the manner previously described. Thirty days after calving the blood was tested again. The color of the milk fat was determined at this time also. The color of the blood serum of another Jersey cow was determined at another time twelve hours previous to the time it was judged she would give birth to a calf. The data obtained in the two experiments are given in Table 7.

TABLE NO. 7.—RELATION OF BLOOD SERUM COLOR TO COLOR OF COLOSTRUM MILK FAT.

Remarks.	Color of serum		Color of fat.	
	Yellow	Red	Yellow	Red
Three days before parturition (Jersey Cow No. 23.)	42.0	0.8		
Thirty days after parturition (Jersey Cow No. 23.)	50.0	2.0	64.0	1.8
Twelve hours before parturition (Jersey Cow No. 2.)	29.0	0.2		

It is readily seen that another explanation must be sought for the high color of colostrum milk fat, other than an accumulation of carotin in the blood. No doubt a certain amount of storing up of carotin does occur if a cow is dry previous to parturition and the serum is low in color at the time of drying up, it being supposed of course that the food contains a plentiful supply of carotin. The data presented in Table 7, when coupled with the data in Tables 3 and 4, show very clearly that under normal conditions the amount of pigment carried by the serum does not exceed a certain maximum point that appears to be practically the same for all cows, regardless of breed. This is not abnormal when it is considered that the carotin of the blood serum is in combination with a protein which no doubt comprises a more or less constant proportion of the blood.

This result forces us to the same conclusion reached in connection with the study of the physiological relation between food, blood serum and milk fat carotin, namely that other factors, among which may be the composition of the milk, must be taken into consideration in explaining the pigmentation of milk fat. In the case of the high color of colostrum milk, some facts stand out that seem to have a special bearing upon the phenomenon. For instance it is a well-known fact that the milk drawn for the first few days after parturition has

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a very abnormal composition which is characterized by a very low fat percentage and a very high protein content, the largest proportion of which is albumin. Unpublished data are at hand which show a composition of some colostrum milks of 1.3 per cent fat and over 4.5 per cent albumin. When it is considered that the carotin is carried by the blood in combination with an albumin, and when it is also taken into account that the source of the lactalbumin is undoubtedly, at least partially, the serum albumin, a most plausible explanation of the high color of colstrum milk fat is at once apparent. It is also apparent that this high color will continue until the milk has reached a normal composition or until the blood supply is depleted. That this will occur regardless of breed is also readily explained since data show that the maximum color of the blood serum does not materially differ with different breeds.

DISCUSSION OF RESULTS.

The results of the foregoing studies in regard to the yellow lipochrome of the blood serum of the cow do not require any extended discussion. Following the interesting discoveries set forth in the preceding papers in regard to the nature of the pigments of milk fat and body fat and their simple physiological relation to the carotin and xanthophylls of the food which the cow receives, it was not surprising to find that the hitherto practically unknown lipochrome of the blood serum of the same animal is also chiefly carotin and bears the same relation to the food as the milk fat carotin. We are thus able to establish the connecting link between the food carotin and the carotin of the milk fat, body fat carotin, corpus luteum, etc., of the cow.

One of the most important results of this study was the discovery that the carotin is not transmitted to the milk glands and body cell from the food by means of simple solution in the blood serum, but is on the other hand carried through the body in combination with an albumin of the serum.[1] This fact is undoubtedly of considerable importance in connection with the entire phenomenon of the pigmentation of the milk fat. It may be safely predicted that all the factors which surround this phenomenon are in some way dependent upon this fact, and all these factors will not be known until it is clearly understood what part this caroto- (or luteo-) albumin

1. Incidentally this discovery has resulted in the addition of a new chromoprotein to the list of conjugated proteins. This is itself of considerable physiological interest.

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plays in the formation of the milk fat. The same holds true for the body fat.

The readily demonstrated fact that the withdrawal of carotin from the food results in a marked decrease in the color of the milk fat being secreted or in the body fat being formed, shows that the albumin which carries the carotin in the blood serum does play a definite part in the formation of both milk fat [1] and body fat and no doubt also in the formation of the corpus luteum.

The whole phenomenon offers many difficult and interesting problems for future study. Many of these when solved will undoubtedly throw light upon the chemistry of the mechanism of milk secretion.

SUMMARY.

1. The well-known lipochrome of the blood serum of the cow is, like the lipochrome of the milk fat, body fat, etc., of the same animal, composed principally of carotin, the widespread hydrocarbon pigment of plants. Associated in small quantity with the carotin of the serum, probably dissolved in the fat of the blood, are one or more xanthophyll pigments, which are always found in more or less variable quantities associated with the carotin of plants.

2. The carotin and xanthophylls of the blood serum are derived from the food and furnish the normal source for these pigments in the milk fat and body fat, etc. A variation in the quantity of these pigments in the food results in a corresponding variation in the amount found in the blood serum and milk fat. Body fat formed during this time will be also affected.

3. The carotin is carried by the blood serum in combination with an albumin. The combination is a very firm one. Lecithin and cholesterol are probably a part of the combination. We propose the name caroto-albumin for this new chromo-protein of the blood.

4. The caroto-albumin of the blood serum of the cow is probably of importance in the formation of the milk fat, body fat and corpus luteum of the cow. It is doubtful if this new pigmented protein is of importance in the oxygen respiration of the body.

5. The lactalbumin of cows' milk may, among other factors, be related to the color of the milk fat. There appears to be a special relation here in connection with the high color and the high albumin content of colostrum milk.

1. The presence of both cholesterol and lecithin in the caroto-albumin may explain the origin of these lipoids, as well as carotin in butter fat.

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B. CAROTIN AND XANTHOPHYLLS DURING DIGESTION

The establishment by us [1] of both a chemical and physiological relation between the carotin and xanthophylls of plants and the yellow lipochromes of the milk fat, body fat, blood serum and corpus luteum of the cow has shown that it is the carotin that is by far the more important in pigmentation of the animal body. It is a well-known fact that xanthophylls are as abundantly and sometimes more abundantly distributed in vegetable matter than carotin. The question naturally arises then, why carotin is the pigment which is principally taken up by the cow's body, and why the xanthophylls appear there only in very small quantity. This seemed to us to be an important physiological question.

It will readily be recognized that a question of this nature is not easily answered. It may therefore be stated in advance that the results of our studies were not as satisfactory as was anticipated. The data are presented, however, for what value they may possess, since opportunity was not presented for a further study of the question. The data are of some value, at least, in that a number of facts are presented which are sufficiently related to advance a fairly acceptable theory in regard to the question.

METHODS OF STUDY

Several methods of study which did not appear to offer many difficulties, seemed available, by which it was thought light could be thrown on the question. One method was to study the action of the various digestive fluids, both natural and artificial, on fresh crude residues of the amorphous carotin and xanthophylls of plants. Another method was to study the nature of the unsaponifiable pigment extracts at various places along the digestive tract of the cow. A third method was closely related to the second and consisted in a study of the unsaponifiable yellow pigments excreted under conditions where unassimilated or undestroyed carotin and xanthophylls of the food would be likely to appear unchanged in the feces. Lack of time made a thorough study of all the methods impossible so that only the significant features of the results of each study will be given.

1. Missouri Agricultural Experiment Station Research Bulletins Nos. 10 and 11, this Bulletin, p. 415 (1914); Jour. Biol. Chem. pp. 191, 211, 223 (1914).

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THE ACTION OF DIGESTIVE JUICES

The following solutions were added to equal portions of carotin and xanthophylls [1] in test tubes, and the tubes plugged with cotton and set aside at 40°C. Observations for signs of decomposition were made every day for five days.

Tube 1. Five cc. of 0.25 per cent HCl solution of pepsin.

Tube 2. Five cc. of 0.25 per cent HCl solution of filtered gastric juice from the fourth stomach of a Jersey cow.

Tube 3. Five cc. of 0.25 per cent Na_2CO_3 solution of trypsin.

Tube 4. Five cc. of 0.25 per cent Na_2CO_3 solution of extract from pancreas of a Jersey cow.

Tube 5. Five cc. of 0.25 per cent Na_2CO_3 solution of trypsin plus 5 cc. of fresh bile from a Jersey cow.

Tube 6. Five cc. of 0.25 per cent NaCO solution of pancreatic extract [2] plus 5 cc. of fresh bile.

1. The carotin and xanthophylls were isolated as follows: 200 grams of air-dried, powdered, green alfalfa leaves were shaken with three litres of 10 per cent alcoholic petroleum ether for two days, and then with 1 litre of CS_2 until the solvent had taken up as much pigment as possible. The carotin and xanthophylls were isolated from each extract and combined. Each solution was now concentrated to 50 cc. and divided into ten parts. These were put into test tubes and the solvent driven off at a low temperature. The residues were used for the studies reported above.

The carotin and xanthophylls were isolated from the alcoholic petroleum ether extract as follows: The xanthophylls were removed from the extract by shaking with an equal volume of 80 per cent alcohol. The carotin in the petroleum ether was now freed from chlorophyll by shaking with an excess of $CaCO_3$, the solution was now evaporated into alcohol and transferred to ether by diluting with much water after the addition of ether. The solution was freed from traces of chlorophyll that had escaped absorption by the $CaCO_3$ by shaking with 30 per cent alcoholic potash. The ether was then freed from alkali with distilled water. This ether solution of carotin was combined with the similar solution obtained from the CS_2 extract as described below. The 80 per cent alcohol, containing the xanthophylls, was partially freed from chlorophyll by shaking with moist animal charcoal for one hour. The pigments were then transferred to ether, the remainder of the chlorophyll being removed by 30 per cent alcoholic potash as in the case of the carotin. The ether solution was then washed free from alkali and added to the xanthophylls obtained from the CS_2 extract as described below.

The carotin and xanthophylls were isolated from the CS_2 extract as follows: The extract was concentrated into 95 per cent alcohol and after filtering was saponified with KOH. The pigments were extracted from the soap with ether. The ether was washed free from alkali and evaporated into alcohol. The carotin and xanthophylls were separated by differentiation between petroleum ether (b. p. 30-50°C.) and the alcohol.

2. The pancreatic extract was prepared by extracting a freshly ground cow's pancreas with 150 cc. of 30 per cent alcohol for 24 hours, straining off the extract, filtering and neutralizing with KOH and 0.5 per cent Na_2CO_3. To prepare the above solution an equal volume of 0.5 per cent Na_2CO_3 was added.

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Tube 7. Five cc. of neutral solution of pancreatin.

Tube 8. Five cc. of neutral pancreatic extract.

Tube 9. Five cc. of neutral pancreatic solution plus 5 cc. of fresh bile.

Tube 10. Five cc. of neutral pancreatic extract plus 5 cc. of bile.

The pepsin, trypsin and pancreatin were Merck's U. S. P. preparations.

A set of ten tubes was also prepared containing equal portions of the xanthophylls of yellow corn.[1]

The following results were obtained. *Carotin*: Bleaching occurred only in the tubes containing neutral and alkaline pancreatic extracts. In the same tubes plus bile there was no decoloration. The bile had no solvent action on the carotin, which was in marked contrast to the xanthophylls, as noted below. *Xanthophylls*: The pigments in tubes 1, 3 and 4 were largely decolorized at the end of the second day, while those in tubes 2, 7 and 8 retained their color after the fifth day. No observations could be made on the tubes containing bile until the fifth day on account of the fact that the bile had completely dissolved the pigments as soon as it was added. The pigments were examined by desiccating the contents of the tubes with plaster of Paris and extracting with ether. Marked bleaching had occurred in all the bile tubes. *Corn Xanthophylls*: There was marked destructive action of these pigments in all the tubes except those containing bile. The corn xanthophylls, like the xanthophylls from the alfalfa, were readily soluble in bile.

The most significant feature of the above results is the marked difference in the solubility of carotin and xanthophylls in bile, the surprising result being the very slight solubility of the carotin. This was confirmed quantitatively using carotin from another source and the bile from several different cows. The results are given in Table 1. The carotin used was a freshly prepared ether solution of carotin from the carrot. Equal volumes of this solution were evaporated at a low temperature and the residues treated with 10 cc. of bile from each of four cows. After standing for several days with frequent shaking the bile was filtered and 5 cc. of the filtrate desiccated with plaster of Paris. This was extracted with ether until colorless. The extract in each case was concentrated to a low volume, made up to 12.5 cc. with absolute alcohol, and the color of the solution measured in the Lovibond Tintometer.

1. This was the unsaponifiable pigment of the corn which was more soluble in 80 per cent alcohol than in petroleum ether.

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TABLE NO. 1.—THE SOLUBILITY OF CAROTIN IN BILE.

Experiment No.	Source of bile	Carotin used		Carotin in bile		Blank*	
		Yellow	Red	Yellow	Red	Yellow	Red
1.	Jersey	57.0	2.0	3.0	0.6	1.0	0.2
2.	Angus	57.0	2.0	9.0	0.8	1.0	0.2
3.	Holstein	57.0	2.0	10.0	0.9	1.0	0.2
4.	Holstein	57.0	2.0	10.5	1.0	1.0	0.2

*The blank is the amount of color extracted from 5 c.c. of bile alone, after desiccation with plaster of Paris.

An interesting feature in the above table is the apparent greater solubility of carotin in the bile of Holstein cows, than in the bile of Jersey cows. If this is confirmed by future study, considerable significance could be attached to it in explaining, at least partly, the differences between the two breeds in the amount of carotin that is secreted in the milk fat.

CHARACTER OF THE PIGMENTS ALONG THE DIGESTIVE TRACT

The plan in this part of the study was to examine the pigments which could be extracted from the material at various places along the digestive tract of several cows. Material was obtained from one Holstein cow and two Jersey cows at slaughtering, from each of the three stomachs just before the food entered the next part of the digestive tract, from three places in the small intestines, from the caecum, and from the large intestine. One or two hundred grams of material were either dried on the steam bath or desiccated with plaster of Paris, and the resulting mass in either case extracted with CS_2. The solubility, spectroscopic, and adsorption properties of the extracted pigments were carefully noted. The pigments were thus differentiated into carotin and xanthophyll constituents as well as classified as belonging to either of the two groups.

The results of the study were not satisfactory, in that there was no uniformity among the several cows in regard to the character of the pigments found at any particular place, although all the animals were receiving a ration which should have furnished an excess of both carotin and xanthophylls. The reason for this is not obvious. It might be thought that the partial drying in some cases destroyed the pigments. Possibly this occurred to some extent, but it would not

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account for the lack of uniformity where this method of desiccation was not employed.

No further discussion will be given this study. Mention has been made of it merely because the method seems to be a valuable one, and will warrant further application.

THE EXCRETED PIGMENTS

For this study the feces of a cow were examined, in a feeding experiment where the carotin and xanthophylls were furnished by the feeding of carrots only. The balance of the ration was composed of grain and timothy hay almost free from carotin and xanthophylls.

The method of demonstrating the character of the pigments in the feces was to desiccate a quantity of fresh feces with plaster of Paris and extract the mass with pure carbon bisulphide. The extract was concentrated and studied spectroscopically, and also by means of a Tswett chromotogramm. The relative solubility properties of the pigments thus found were studied, and also the spectroscopic properties of the pigments thus separated.

In this way it was found that when the cow was receiving 50 pounds of carrots per day, both carotin and xanthophylls were abundantly present in the feces. This continued for six days after the carrots were withdrawn from the ration, although it was possible to detect but little xanthophyll during this time.

DISCUSSION OF RESULTS

Combining the results of the above experiments, the appearance of carotin in the cow's system when fed in excess may be explained on the ground of its greater stability toward the digestive processes, as shown by the digestion experiments, and the abundant appearance of the pigment in the feces. The failure of the xanthophylls to appear to any extent in the cow's system may be due similarly to the fact that they are apparently more easily destroyed [1] during digestion. Some of them that escape destruction are undoubtedly taken up by the bile and thus enter the system through the portal circulation. Some oxidation probably takes place in the liver. If fat is present to any extent some of the xanthophylls will evidently be taken up and

1. Willstätter and Mieg. (Ann. d. Chem. 355, p. 1, 1907), state that xanthophylls are very sensitive toward acids. This would lead one to expect that they would be largely destroyed by the gastric juice. Our results were contradictory in this respect. We found an artificial gastric juice to destroy the xanthophylls but the natural gastric juice from the fourth stomach of a cow apparently had no effect on them.

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enter the blood dissolved in fat. In this connection it is of interest to recall that we have shown[1] that there is evidence to indicate that what xanthophylls can be found in the blood are present dissolved in fat.

An additional possible explanation of this whole question should not be overlooked, however, namely, that the difference in the proportion of carotin and xanthophylls taken up by the cow's body may be due entirely to the difference in chemical composition between carotin and xanthophylls. Carotin is an unsaturated hydrocarbon and is furthermore capable of combining with a protein of the blood, as we have shown.[2] The xanthophylls, on the other hand, are carbon, hydrogen and oxygen compounds, in fact are chemically carotin-dioxides. Although still unsaturated bodies, their slight difference in composition from carotin, may prevent their combination with the serum albumin, thus making it impossible for them to appear to any extent in the blood and fatty formations of the cow's body. If fat played a greater part in the food of the cow, the xanthophylls would undoubtedly appear to a greater extent in the body of this animal.

SUMMARY

1. Carotin is assimilated from the food of the cow in preference to xanthophylls partly because of its greater stability toward the juices of the digestive tract. Xanthophylls are much more soluble in bile than carotin,[3] which probably accounts for their appearance in the fat of the blood.

2. It is probable that carotin forms by far the greater part of the lipochromes of the cow's body chiefly on account of its ability to form a compound with one of the proteins of the blood. The xanthophylls, being of different composition, probably are not capable of forming such a compound.

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1. This Bulletin, page 422; Jour. Biol. Chem. 17, p. 211, 1914.
2. *Ibid.*
3. A confirmation of the very slight solubility of carotin in bile is seen in the recent finding of Fischer and Röse (Zeit. f. Physiol. Chem. 88, p. 331, 1913), that the gall stones of cows contain crystallizable carotin. No xanthophylls were found.

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C. THE PIGMENTS OF HUMAN MILK FAT

The discovery of the chemical and physiological relations of the pigments of the fat of cow's milk to the carotin and xanthophylls of plants naturally opens the question whether the pigments which characterize the fat of other animals are of a similar character. Opportunity was not afforded to study this question with any domestic animals other than the cow. An opportunity, however, was offered to investigate the character of the pigments which sometimes give a high color to the fat of human milk.

The methods used for studying the character of the pigments were the microscopic ones used in the preceding studies. The adsorption properties were not studied, however, the demonstration being confined to the observation of the absorption bands and the relative solubility properties.

The fat from two samples of human milk from different sources was used. Very little was known in regard to one of the samples, it having been sent to the laboratory for analysis by a well-known physician of the community. The other sample was taken by one of us from a woman who had just given birth to a child, and represented a portion of the milk of each day of the first few days of lactation. Some further observations in regard to this sample will be reported below.

Experiment No. 1

This was the sample in regard to which very little was known, with the exception that it was a bona fide sample of human milk. The milk had a faint yellow tint. The volume of milk used was approximately 125 c.cm. The milk contained about 3.5 per cent fat and therefore yielded a little over 4 grams of fat. The fat was obtained from the milk by precipitating it along with the proteins. To do this the milk was acidified with acetic acid, a pinch of salt added, and the milk brought to a boil. The precipitated proteins, when filtered off, had a bright yellow color, due to occluded fat. The fat was dissolved out with hot 95 per cent alcohol.

After concentrating the alcoholic extract, the fat was saponified by adding a small piece of KOH and boiling for about one hour. The pigment was readily extracted from the soap by ether, after dilution with water. The golden-yellow ether solution was washed with water and evaporated to dryness. The residue dissolved at once in carbon bisulphide with a red-orange color and in this solution

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showed two beautiful absorption bands, and possibly a third. The CS_2 was carefully evaporated. A part of the residue which remained was difficultly soluble in absolute alcohol, but readily dissolved when a little petroleum ether was added. When differentiated between petroleum ether and 80 per cent alcohol the combined pigment was readily divided into two apparently equal proportions with perhaps slightly more color in the petroleum ether layer.

The pigment of the petroleum ether layer gave a red-orange carbon bisulphide solution showing two strong absorption bands and a third faint one, the measurements of which are given in Table No. 1 below.

TABLE NO. 1.—ABSORPTION BANDS OF CAROTIN AND XANTHOPHYLLS FROM HUMAN MILK FAT.

Experiment.	Measurements of absorption bands.	
	Carotin	Xanthophylls.
No. 1	I. 225—244 II. 262—280 III. 300—319	I. 234—253 II. 275—293 III. 320—
No. 2	I. 225—242 II. 265—282 III.	I. 232—252 II. 273—293 III. 312—330

The pigment of the alcoholic layer gave a yellow-orange, carbon bisulphide solution showing two good absorption bands and end absorption, the measurements of which are given in Table 1.

Experiment No. 2

As stated above, this sample of human milk was taken by one of us and represented the milk of the first few days of lactation including the colostrum milk. The milk itself was characterized by a high yellow color and the fat which rose to the top of the sample had a very deep yellow color. About 350 c.cm. of milk were obtained. The fat percentage being between 5 and 6, nearly 20 grams of fat were yielded for the study of the pigments.

The fat was obtained from this sample of milk in a manner very similar to that used in the preceding experiment. The proteins and fat were precipitated together by adding a little salt and also con-

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siderable ammonium sulphate, acidifying with acetic acid and bringing to a boil. The precipitate was filtered off on a Büchner funnel. The layer of protein and fat had a golden-yellow color. The fat was extracted with hot alcohol and ether. The golden-colored extract was evaporated to dryness and the fat dissolved away with ether. Alcohol was added and also 5 grams of KOH and saponification of the fat allowed to proceed on the steam bath for one-half hour. The pigment was extracted from the diluted soap with ether. After thorough washing with distilled water, the ether was evaporated carefully to dryness. The residue had a deep red color. It dissolved at once in petroleum ether (b.p. 30°-50°C.).

The pigment in this solution was now differentiated between the petroleum ether and 80 per cent alcohol. In this way it was divided into two portions which were about equal as far as could be detected by the color of the two solutions, with perhaps slightly more color in the 80 per cent alcohol.

The pigment in the petroleum ether layer gave a blood-red colored carbon-bisulphide solution which showed two absorption bands and considerable end absorption. The measurements of these bands are given in Table 1.

The pigment in the 80 per cent alcohol layer gave an orange-colored carbon-bisulphide solution which showed three distinct absorption bands. The measurements of these bands are given in Table No. 1.

DISCUSSION OF RESULTS

The results of the above experiments show very clearly that the fat of human milk may be tinted with the same pigments found in the fat of cow's milk. The relative proportion of carotin and xanthophylls in human milk fat is much more nearly equal than in the fat of cow's milk. This is not surprising when it is considered that there is strong evidence that the xanthophylls are conveyed through the body dissolved in fat, and when it is also considered that fat plays a much greater part in human food than in the food of the cow.

An especially interesting fact brought out by these brief studies is that colostrum milk fat of the human is characterized by a very high color just as is the case with the fat of the colostrum milk from cows. In the experiment here reported, one of us had occasion to observe that after about ten days the milk fat from the same woman was very much lighter in color than during the first few days of lactation. The milk was also observed at intervals for a period of

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several months. Considerable variation in the color of the fat was noticed. Although it was not possible to accurately trace the cause of this variation, as we did in the case of cows in an earlier paper of this series, it was undoubtedly due to changes in diet.

In conclusion it may be stated that all students of human anatomy are familiar with the fact that the fat on the human body is often characterized by a marked yellow color. In view of the fact that the pigments of the milk fat and body fat of the cow are identical, it must therefore be concluded that the pigments of the milk fat and body fat of humans are identical.

SUMMARY*

1. The fat of human milk may be tinted by carotin and xanthophylls, the pigments which characterize the fat of cows' milk. The relative proportion of carotin to xanthophyll in human milk fat is much more nearly equal than in the fat of cows' milk.

2. The colostrum fat of human milk is characterized by a very high color as is the case with the fat of the colostrum milk of cows.

3. The pigment of human body fat is no doubt identical with the pigment of human milk fat.

*See page 438 for summary of "The Yellow Pigment of Blood Serum."
See page 446 for summary of "Carotin and Xanthophylls During Digestion."

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RESEARCH BULLETIN NO. 13

UNIVERSITY OF MISSOURI

COLLEGE OF AGRICULTURE

AGRICULTURAL EXPERIMENT STATION

LACTOCHROME—THE YELLOW PIGMENT OF MILK WHEY

Its Probable Identity With Urochrome, the Specific Yellow Pigment of Normal Urine.

COLUMBIA, MISSOURI
April, 1914

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CO-OPERATION.

The investigation here reported was conducted by the Missouri Agricultural Experiment station in co-operation with the Dairy Division of the United States Department of Agriculture, Washington, D. C.

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LACTOCHROME—THE YELLOW PIGMENT OF MILK WHEY

Its Probable Identity With Urochrome, the Specific Yellow Pigment of Normal Urine.

LEROY S. PALMER AND LESLIE H. COOLEDGE.

The natural yellow color of cows' milk is caused by two entirely different kinds of pigments. The principal one of the two pigments is found in the milk fat and causes the more or less yellow color of cream and butter. This pigment has recently been found to be identical with carotin,* the widely distributed orange-red hydro-carbon vegetable pigment found in the carrot and which is also found accompanying chlorophyll in all green plants.

The secondary or minor pigment of milk has not heretofore been identified. Its presence in the milk is largely masked by the white color of the caseinogen and is only seen in the whey which remains after coagulation of the caseinogen. The pigment is then seen imparting the usual greenish-yellow color to the resulting whey. The same greenish-yellow color also characterizes the so-called rennet whey and is a familiar phenomenon to the cheese maker.

The object of the present investigation was to study the importance of the whey pigment in the pigmentation of milk and if possible to establish its relation to other well known animal pigments of similar character.

HISTORICAL

The yellow color of milk whey was observed as long ago as 1784 by Schoepff,[1] "Liquidem colore diluti citrinum." Nearly one hundred years later Blyth[2] in studying the so-called lacto-protein which Millon and Cammille[3] isolated from cows' milk, separated it into a protein which he called galactin and a pigment which he called lactochrome.[4]

*"Carotin—The Principal Natural Yellow Pigment of Milk Fat," by L. S. Palmer and C. H. Eckles. Missouri Agricultural Experiment Station Research Bulletin No. 10; Jour. Biol. Chem. 17, p. 191 (1914).

1. Schoepff, "Specimen Inaugurale chemico-medicum de Varus Lactis Buli Salibus alusque Substantius in ejusdem parte Aquosa Contentis, etc." Quoted from Blyth, "Foods, Their Composition and Analysis."
2. Trans. Jour., London Chem. Soc. p. 530, 1879.
3. Comp. Rend. 59, p. 301 (1864).
4. The method of isolation is given on page 455.

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Blyth obtained the pigment as a mercury salt and finally in the free state. "As obtained in this way it was in the form of a bright red-orange, resin-like mass, softening at 100° C, very soluble in hot alcohol but partially separating out on cooling. The concentrated solution gave a simple spectrum allowing most of the red and yellow rays to pass through, and no bands were observed." Blyth analyzed the mercury salt and gave it the formula $HgOC_6H_{18}NO_6$. It seems very probable that Blyth was dealing with the specific milk whey pigment.

Reference to the pigment lactochrome is occasionally found in textbooks but in most cases erroneously in connection with the color of the milk fat. Blyth [1] himself says, "Milk fat under the form of butter is constantly tinted more or less yellow from dissolved lactochrome."

Lewkowitsch [2] states that, "Pure butter fat —— contains cholesterol and some natural coloring matters (lactochromes)." Oliver [3] says, "Lactochrome is probably derived from the haematin of the blood, is of a resinous character, of a bright orange color, and is soluble in water. This causes the color of butter and of whey. It is present in very small proportions and varies much in that respect according to the action of the alveolus cells." Wing [4] in his latest text book states that lactochrome gives to milk its characteristic color, and that it is associated principally with the palmitin of the butterfat. He also states that the amount of lactochrome in the milk varies with the breed of the animal and the character of the food.

Desmoulière and Gautrelet [5] recently studied a greenish-yellow pigment which they isolated from milk whey and from their investigation felt justified in concluding that it was identical with urobilin.[6] According to these authors if the slightly cloudy greenish-yellow milk whey is acidifed with sulphuric acid and saturated with ammonium sulphate the greenish-yellow color is entirely precipitated along with the proteins; and the precipitate will yield to 90 per cent alcohol a yellow pigment with a greenish fluorescence, which, after acidifying,

1. Text "Foods, Their Composition and Analysis," 1896, 4th edition, p. 239.
2. Oils, Fats and Waxes, Vol. II, p. 667 (1909 edition).
3. "Milk, Cheese and Butter," p. 44.
4. "Milk and Its Products," 1912 edition.
5. Compt. rend. soc. Biol. 55, p. 632 (1903); cited in "The chemistry of Milk," by Kastle and Roberts, Hygienic Laboratory, Bul. No. 56, p. 319.
6. This is the pigment that is found in small quantities in normal urine and which gives to urine its characteristic spectrum of a single absorption band in the neighborhood of the F line.

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will show the characteristic absorption band of urobilin. In addition the pigment is claimed to have been isolated in the free state as rusty colored flakes, difficultly soluble in water.

In order to show that the pigment which they isolated pre-existed in the milk and was not converted into urobilin during the process of isolation, the investigators saturated a volume of milk with ammonium sulphate and added one half its volume of 90 per cent alcohol. The solution was shaken and let stand until a clear separation was obtained. The alcoholic layer had extracted the greenish-yellow color of the whey. The authors do not state that the yellow extract thus obtained showed the absorption band of urobilin. The conclusion that the pigment extracted in this way was urobilin seems to have been based on the grounds that this extraction test is a characteristic one for urobilin. The authors, in addition, emphasize the fact that the isolated pigment did not give the characteristic color reactions of a lipochrome with concentrated sulphuric and nitric acids. They accordingly conclude that no lipochromes exist in milk. The absurdity of the conclusion is apparent. It seems likely that these investigators as well as Blyth were dealing with the specific milk whey pigment.

The two investigations reported above, i. e. the one by Blyth and the one by Desmoulière and Gautrelet, are the only ones which the literature report in connection with the whey pigment. It is clear that both investigations concern only a pigment of the whey, notwithstanding the erroneous allusion in one case to the pigment of butter and in the other to lipochromes. In addition it is readily seen that the question is far from being satisfactorily settled. If Blyth was dealing with the true pigment, his work is unsatisfactory in that no attempt was made to show its relation to any of the known animal pigments. If the results of Desmoulière and Gautrelet are correct and the whey pigment is urobilin then both investigations would seem to require repetition to see whether they were dealing with entirely different bodies, and if so to determine which is the specific whey pigment. At the same time the apparently contradictory results of the two investigations could be satisfactorily explained.

THE ISOLATION OF BLYTH'S LACTOCHROME

Lactochrome was isolated from milk according to Blyth's method as follows: 1000 cubic centimeters of separated milk were divided into three portions. One of these portions was diluted with water to about four times its original volume and acidified with dilute acetic acid until the casein coagulated. The whey was then syphoned off

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into a second portion of the milk and more acid added if necessary. The resulting whey was in its turn syphoned into the third portion of the milk. More acid, if necessary, was added and the casein filtered out. This solution, free from casein, was now raised to the boiling point and gently boiled for several minutes. The albumin and globulin coagulated and were easily filtered off. The greenish-yellow whey was then precipitated with acid mercuric nitrate solution.* The dense precipitate which formed was washed by decantation and suspended in a small amount of water. It was then decomposed with hydrogen sulphide gas, after acidifying slightly with either nitric or hydrochloric acid. The resulting mercuric sulphide was filtered off and the excess hydrogen sulphide removed from the filtrate with the aid of heat. An excess of lead acetate was added, which threw down the dirty white precipitate which Blyth called galactin. This was filtered off and the excess of lead acetate in the filtrate decomposed with hydrogen sulphide. The resulting lead sulphide was filtered off, the filtrate being more or less yellow in color depending upon the amount of lactochrome in solution. The lactochrome in this solution was purified by reprecipitation several times with mercuric nitrate, decomposing the final mercury salt with hydrogen sulphide, evaporating the aqueous solution to dryness, and dissolving the residue in hot 98 per cent alcohol. This alcoholic solution was considered to be practically pure lactochrome.

The maximum yield obtained by this method was about 0.04 gram per liter of milk. Some of the pigment was lost with each purification. About 0.5 gram in all was obtained for the following study.

The lactochrome, as obtained, was an amorphous orange colored substance with a peculiar odor. It conformed in every particular to the description given by Blyth. It showed no spectroscopic absorption bands either in aqueous or alcoholic solution when the solutions were neutral or after they had been acidified with hydrochloric acid.

The pigment was precipitated as a yellow salt from both its alcoholic and aqueous solutions by phosphotungstic and phosphomolybdic acids, and by mercuric nitrate, silver nitrate and lead acetate, the lead salt being soluble in an excess of lead acetate. Lactochrome was not precipitated by lead acetate from its solution in the presence of either

*Note:—The mercuric nitrate solution was made by dissolving 100 gms. of pure mercury in one liter of concentrated HNO_3, a further quantity of acid was added until no red fumes were evolved; the solution was evaporated to a syrup and after adding enough HNO_3 to prevent the formation of a basic salt the solution was made up to 1400 c.c. with distilled water.

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nitric or hydrochloric acid which is the principle upon which is based the separation of the "galactin" from the lactochrome by lead acetate.

Lactochrome gave a greenish colored copper salt on the addition of a neutral solution of copper acetate. This salt was readily soluble in NH_4OH. The lead and mercury salts were readily soluble in ammonium acetate solution. Very little ammonium acetate was necessary to prevent the precipitation with lead acetate; it required more, however, to prevent the precipitation of the mercury salt.

The yellowish-brown residue which was left on evaporation of the alcoholic solution, imparted a faint color to chloroform which seemed to increase on the addition of a drop of concentrated NH_4OH. Alcoholic zinc chloride when added caused no green fluorescence. Amyl alcohol seemed to extract a slight amount of color from the dried residue, the amount of color absorbed seeming to increase on standing. This was probably due to absorption of water from the air,—the pigment being very soluble in water. The amyl alcohol solution showed no absorption bands after acidifying with HCl when viewed in front of the spectrometer through a 10 mm. layer, although the solution showed a good yellow color at this depth.

Lactochrome was only very slightly precipitated on saturation of its aqueous solution with ammonium sulphate. A scant brown precipitate was thrown down in this way but the filtrate had apparently retained all its former color. On addition of an equal volume of absolute alcohol to this filtrate and mixing thoroughly, the alcohol rose to the top with practically all the color.

Lactochrome was found to be readily soluble in milk whey, a small amount appreciably increasing the original color of the whey.

THE ISOLATION OF UROBILIN FROM MILK ACCORDING TO THE METHOD OF DESMOULIERE AND GAUTRELET

In order to demonstrate the presence of urobilin in milk, six liters of skim milk were treated as follows according to the method of Desmoulière and Gautrelet: The casein and fat were precipitated by acidifying with acetic acid at a temperature of 50°C. After filtering off the casein, the slightly cloudy serum had a pronounced yellow color. The acetic acid in the serum was neutralized with NH_4OH and the serum made acid with H_2SO_4 and saturated with solid ammonium sulphate. After standing for several hours the heavy precipitate was filtered off.

Contrary to the observations of Desmoulière and Gautrelet the filtrate was not colorless but was apparently as high colored as before

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saturation with ammonium sulphate. That the precipitate contained some pigment, however, was shown by the fact that after careful washing with a saturated solution of ammonium sulphate and drying in the steam oven, it gave up a greenish-yellow extract to 90 per cent alcohol. On evaporating off the alcohol from this extract, leaving the pigment in aqueous ammonium sulphate solution, only a small part of the pigment was re-precipitated, the bulk of the coloring matter being again found in the filtrate. An alcoholic solution of the portion of the pigment which had been precipitated the second time was obtained as before by washing with saturated ammonium sulphate solution, drying, and extracting with 90 per cent alcohol. The golden-yellow green-fluorescent solution of about 25 c.c. volume, which was obtained in this way, was tested for urobilin properties. The solution, however, showed no absorption band in either neutral or acid (HCl) solution. On evaporation of the solution the residue was somewhat soluble in chloroform giving a yellow solution with a slight green fluorescence. The addition of ammoniacal alcoholic zinc chloride to the chloroform solution gave no indication of the beautiful green fluorescence of Wirsing's urobilin reaction, and the solution showed no absorption bands. The coloring matter was completely precipitated from both aqueous and alcoholic solutions by mercuric nitrate and silver nitrate.

The result of this experiment, the principal features of which were confirmed by numerous other investigations of the same nature, showed that the claims of Desmoulière and Gautrelet in regard to the presence of urobilin in milk cannot be confirmed. In addition, they led to an investigation of the pigment which in such large measure remained in solution on saturation with ammonium sulphate. As was stated above, Desmoulière and Gautrelet apparently based their conclusion that urobilin is present in milk partly on the ground that alcohol will extract a yellow color from whole milk which has been saturated with ammonium sulphate. Attention was accordingly directed to the extraction by this method of the pigment which we found was not precipitated on saturating the acid whey with ammonium sulphate. It was found on adding an equal volume of absolute or 98 per cent alcohol to the highly colored ammonium sulphate saturated serum under investigation above, that after thorough mixing, the alcohol rose to the top in a clear greenish-yellow layer, carrying with it practically all the pigment that was in the serum. Numerous attempts to make the pigment of this extract exhibit spectroscopic absorption properties resulted in failure. On the other hand the pigment exhibited all the properties which had been found to be characteristic of lactochrome. In this

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connection attention was especially drawn to the facts that neither pigment was precipitated to any extent on saturation of its aqueous solution with ammonium sulphate, and that both pigments on isolation failed to show any spectroscopic properties. (The latter was also true of the small portion which was precipitated from the fresh whey with ammonium sulphate.)

On studying the above mentioned properties of the whey pigment isolated as either lactochrome or by the alcohol extraction method, there seemed to be a remarkable similarity to the properties of urochrome, the specific urinary pigment. Hammarsten [1], summarizing the properties of urochrome, describes the isolated pigment as, "Amorphous, brown, readily soluble in water and ordinary alcohol, but less soluble in absolute alcohol. It dissolves but slightly in acetic ether, amyl alcohol, and acetone, while it is insoluble in ether, chloroform, and benzene. Urochrome is precipitated by lead acetate, silver nitrate, mercuric acetate, phosphotungstic and phosphomolybdic acids. On saturating the urine with ammonium sulphate a great part of the urochrome remains in solution. It does not show any absorption bands and does not fluoresce after the addition of ammonia and zinc chloride."

In addition Hammarsten states that, "Urochrome can be prepared according to a rather complicated method which is based upon the fact that the substance remains in great part in solution on saturating the urine with ammonium sulphate. If the proper quantity of alcohol is added to the filtrate, a clear, yellow alcoholic layer forms on the salt solution, which contains the urochrome and which can be used for the further preparation of the latter."

It is especially striking that the whey pigment lends itself to isolation in exactly the same way. It might be stated here that the property of being extracted by alcohol from a solution saturated with ammonium sulphate is a urochrome characteristic and not a urobilin characteristic as Desmoulière and Gautrelet believed in connection with their work on "Urobilin" in milk.

Up to this point it was concluded that it has been satisfactorily demonstrated that the whey pigment is not urobilin, that the whey owes its characteristic color to the pigment which Blyth called lactochrome; and that the pigment could be isolated by means of a method identical with the one which was used by Garrod [2] to isolate urochrome from

1. Hammarsten-Mandel, "Text book of Physiological Chemistry," p. 703, 6th American edition 1911.
2. Proc. Roy. Soc. 55, p. 394 (1904).

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urine. In addition the pigment which for the present may be called lactochrome, was characterized by a large number of properties identical with those of the pigment urochrome. In fact the resemblance was so strong that the investigation of the lactochrome was further pursued with a view of establishing its relation to the specific urinary pigment.

THE STUDY OF THE UROCHROME PROPERTIES OF THE WHEY PIGMENT

It became necessary before proceeding with the investigation, to study more closely the properties of urochrome in order to select some properties which could be applied to the pigment lactochrome, the result of which would be to demonstrate beyond a doubt the identity of the two pigments.

The most desirable means of identification would have been a study of the elementary composition. This was abandoned, however, partly because of the great difficulty attending the isolation of the pigment in a degree of purity sufficient for elementary analysis, but chiefly on account of the fact that none of the investigations of the elementary analysis of urochrome which have already been made have been found to agree. For instance, Dombrowski [1] found 11.15 per cent nitrogen, Holweg [2] found 9.89 per cent nitrogen, while Klemperer [3] found only 4.2 per cent nitrogen. Again Dombrowski,[4] basing his method upon the fact that urochrome readily forms a copper salt with copper acetate solution, isolated a product containing varying amount of sulphur up to six per cent, while Holweg [5] using a method which did not involve the use of any metallic precipitants, found that urochrome was free from sulphur. Salomonsen,[6] using Holweg's method, found that the product contained plenty of sulphur and in addition contained, "Considerable iron which could not be removed by repeated solution in alcohol and precipitation with ether." Mancini [7], also using Holweg's method, found that the substance contained sulphur but that its union with the urochrome molecule was very unstable.

1. Zeit. f. Physiol. Chem. 54, p. 188 (1908).
2. Biochem. Zeit. 13, p. 199 (1908).
3. Ber Klin. Wochensch. 40, No. 14 (1903).
4. Loc. cit.
5. Loc. cit. Holweg evaporated the urine to a syrup, extracted the urochrome with animal charcoal and after washing, extracted the pigment from the animal charcoal with glacial acetic acid. The urochrome was obtained in the free state from its solution in glacial acetic acid by precipitation with ether or by evaporation of the acetic acid in vacuum.
6. Biochem. Zeit. 13, p. 205 (1908).
7. Biochem. Zeit. 13, p. 208 (1908).

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Garrod [1], studying urochrome in relation to other animal pigments, found that an alcoholic urochrome solution after treatment with "active" acetaldehyde exhibited a great many of the characteristic properties of urobilin including the characteristic urobilin absorption band and the production of the usual green fluorescence on the addition of ammoniacal zinc chloride solution. At the same time, the "artificial" absorption band showed the position which characterizes the ordinary urobilin band after the same treatment. According to Garrod, this reaction is a very delicate one, "A solution of one part of urochrome in 30,000 yielding the reaction quite definitely, although such a solution appears colorless in depths of two or three centimeters."

Dombrowski [2] failed to confirm the action of "active" aldehyde on the urochrome prepared by him, no bands whatever being formed. He considered his aldehyde solution "active," however, because it restored the optical properties of a urobilin solution whose absorption band had been destroyed by evaporation of its aqueous solution in the presence of ether,[3] or by oxidation with potassium permanganate.[4] In these cases, however, the position of the band was not in the same place in which it was found before.

Holweg also tried to apply Garrod's aldehyde test to the urochrome isolated by him, with the result that the urochrome solution, "After a short treatment with pure acetaldehyde in the warm and subsequent addition of ammoniacal zinc chloride solution, showed an extraordinary strong green fluorescence, not at once, but after standing forty-eight hours in the air. At the same time no characteristic urobilin band occurred in the blue but only a diffuse absorption of the stronger refracted rays of the spectrum."

One of the few tests for urochrome which all investigators of the pigment have confirmed is the fact that it gives the reaction for pyrrol when heated with zinc dust or KOH.

Lately Salomonsen [5] and also especially Mancini [6] have shown that urochrome will give a bromine derivative which they claim is a very characteristic compound and which may be used for the identification of the pigment.

1. Jour. Physiol. 21, p. 190 (1897); 29, p. 335 (1903).
2. Loc. cit.
3. Method of Hopkins and Garrod—Jour. Physiol. 29, p. 339 (1903).
4. Method of Riva and Chiodera—Arch. Ital. d. chem. med. 35, p. 505 (1896).
5. Loc. cit.
6. Loc. cit.

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After a careful review of the above investigations especially the ones concerning the aldehyde reaction and the bromine compound, it was concluded that they were sufficiently characteristic to apply them to the whey pigment and thus definitely establish its relation to urochrome. It will not be necessary to discuss the pyrrol reaction or the formation of the bromo-urochrome further at this point In view, however, of the contradictory results obtained by Dombrowski and Holweg with the "active" aldehyde reaction, this reaction seems to deserve some consideration.

A close study of Garrod's paper setting forth the action of "active" aldehyde on alcoholic urochrome solutions, will lead to the conclusion that Garrod's results cannot well be doubted. Certainly the possibility of the presence of urobilinogen in his solutions is precluded, on the ground of the method of preparation and also on the ground of the existence of a number of characteristic properties which accompany the reaction. The method of preparation itself would preclude urobilinogen if we are to believe the text book statements, that that inert body is precipitated on saturation with ammonium sulphate; and chief among the accompanying properties is to be considered the development of a second very characteristic absorption band in the blue on continuing the action of the aldehyde upon the urochrome, especially since no such band developed on allowing the aldehyde to act for a corresponding period of time upon a natural urobilin solution which already showed the normal urobilin band. Again, Garrod found that the urobilin which resulted from the action of the aldehyde resembled other artificial urobilin-like products in that the redward border of the band was less distinct than the natural urobilin band, and that the product did not give the E band spectrum which is given by natural urobilin on neutralizing or slightly acidifying an alkaline urobilin solution with sulphuric acid. On the other hand the artificial urobilin resembled natural urobilin in many respects. The action of the aldehyde changed the original yellow color of the urochrome to a deep orange tint and on dilution this color changed to the characteristic pink color of dilute urobilin solutions. Ammonia changed the deep orange color to a pale yellow with the disappearance of the absorption band. On addition of a little zinc chloride solution to the ammoniacal solution the characteristic brilliant green fluorescence of a natural urobilin solution appeared and also the shifted absorption band. Garrod gives the following measurements of the absorption bands.

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	Neutral Alcoholic solution	Ditto with zinc chloride and NH_4OH
Product from urochrome	λ 5150-4860 shading to 4550	λ 5170-4990 shading to 4770
Urobilin from urine	λ 5110-4810 shading to 5590	λ 5170-4990 shading to 4770

The urochrome product further resembled natural urobilin in being precipitated on saturation of its aqueous solutions with ammonium sulphate.

On allowing the action of the aldehyde to continue upon the urochrome, Garrod found that the color of the solution changed to a deep red brown and a second absorption band appeared.

Garrod measured the two bands at this stage as follows:

Band A λ 5130-4910

Band B λ 4720-4570

The substance which gave the second band was extracted along with the urobilin-like band with chloroform, but after repeated washings with water the chloroform showed only Band B while the washings showed Band A. After evaporation of the resulting chloroform solution, an alcoholic solution of the residue showed Band B with great distinctness.

As a final proof of Garrod's results is to be mentioned the fact that similar results were obtained with a urochrome solution which had been obtained by an entirely different method,[1] namely by the method devised by Kramm.[2] This method was similar to the one used by Holweg[3] and consisted in extracting the urochrome from the urine by animal charcoal and, after washing and drying in vacuum, extracting the pigment from the animal charcoal by means of a phenol-alcohol solution. The above especial consideration of Garrod's "active" aldehyde reaction has been given because, as previously stated, the work seemed to us of such high character and the reaction so characteristic that it would certainly lend itself, if rightly carried out, to an ultimate solution of the identity of the milk whey pigment. That this proved to be the case will be seen on consideration of the results obtained.

1. Garrod—Jour. Physiol. 21, p. 191 (1897).
2. Deut. med. Wochenschr. 22, pp. 25 and 42 (1896).
3. Loc. cit.

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THE ACTION OF "ACTIVE" ACETALDEHYDE ON UROCHROME AND LACTOCHROME

For the study of the action of "active" acetaldehyde, fresh quantities of urochrome and lactochrome were prepared as follows: The preparation of the "active" aldehyde is also described.

PREPARATION OF UROCHROME

The urochrome was isolated according to Garrod's [1] method as follows: About eight liters of well colored normal human urine were concentrated to about 250 c.c. on the steam bath, and the syrupy fluid filtered. The filtrate was saturated with ammonium sulphate in substance and the resulting precipitate filtered off. The filtrate was clear. It had a deep red color, but a small portion of it on dilution passed through a golden to a pale greenish-yellow color.

An equal volume of 98 per cent alcohol was now added to the filtrate, precipitating considerable ammonium sulphate. After mixing thoroughly and letting stand for a few minutes, the alcohol layer rose to the top as a highly colored solution. The alcohol layer was separated and fresh alcohol added to the lower aqueous layer. More pigment was extracted. The alcohol extracts were combined and the resulting solution of about 300 c.c. volume poured into 500 c.c. of water. The alcohol layer was made to separate from this solution by adding ammonium sulphate with the aid of gentle warmth. The alcohol extract obtained in this way was poured upon some solid ammonium sulphate and gently warmed. Two layers again formed, and the alcohol layer again separated.

The final alcohol extract was now evaporated nearly to dryness at a low temperature keeping the reaction of the solution alkaline with NH_4OH, and the evaporation finally was carried to dryness in vacuum. A brown-red residue remained which softened on warming but was nearly solid when cold. It was washed twice with acetic ether to remove indoxyl sulphate and was then allowed to soak over night in absolute alcohol, in a glass stoppered bottle. The alcoholic extract was filtered off and the residue treated with fresh absolute alcohol. The combined extracts were poured into several volumes of anhydrous ether, precipitating most of the pigment as a sticky brown-colored substance which clung to the bottom and sides of the beaker. The alcohol-ether solution was poured off and the precipitated pigment

1. Proc. Roy. Soc., 55, p. 394 (1894).

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dried in the vacuum oven. It was then allowed to stand under chloroform for about one hour which extracted a little color; and after draining off as much of the chloroform as possible the remainder was driven off in vacuum.

Three extracts were obtained from the residue, an absolute alcohol extract, a 98 per cent alcohol extract from the residue left after the absolute alcohol extraction, and an aqueous solution of the residue left after the two alcohol extractions. These were all considered to be fairly pure urochrome solutions and the three extracts were used in the studies to be reported later.

ISOLATION OF THE MILK WHEY PIGMENT

The isolation of the milk whey pigment was found to be rather difficult on account of the fact that the pigment so readily decomposes in aqueous solution, especially in its solution in the serum.* A beautiful greenish-yellow casein and albumin-free serum will sometimes almost entirely bleach out on standing over night. The alcoholic solutions are much more stable, however, and in this respect are identical with urochrome. The conditions surrounding the decomposition of the whey pigment are not well defined, the bleaching occuring as well in neutral as in acid solution. Again it has been found possible to carry out the entire tedious procedure of isolation with no indication of the bleaching. These remarks seem pertinent at this point because they emphasize the fact that ordinarily the isolation required that it be carried out with the utmost speed to the point where the pigment is brought into alcoholic solution.

The method which proved fairly successful and which was used in the isolation of the pigment for some of the following studies was a combination of Blyth's method for the isolation of lactochrome and Garrod's method for the isolation of urochrome.

Skim milk, which a preliminary test indicated would yield a well colored serum, was acidified with sufficient acetic acid to coagulate the casein and, after filtering, the cloudy acid whey brought to boiling and boiled for several minutes. The clear, hot filtrate from this coagulation was now neutralized with sodium hydroxide solution and the further coagulation which occurred removed by filtration. Mercuric nitrate was now added in excess and the cream colored

*Freudenreich (Land. Jahrb. Schweiz. 14, p. 51, 1900), noticed that milk serum containing 5 per cent lactic acid had a higher yellow color than the same milk containing 1 per cent lactic acid.

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precipitate which formed removed by filtering on a Büchner funnel, using suction. The precipitate on the filter was suspended in water and thoroughly washed and then filtered off again on the Büchner. This treatment was repeated several times, thereby removing all water soluble impurities including the lactose, excess mercuric nitrate, etc. After the final washing and filtering, the precipitate was sucked quite dry and then removed from the funnel and rubbed up with water in a mortar to a fine suspension and decomposed with a stream of hydrogen sulphide. The mercury sulphide which formed was separated by acidifying with HCl and boiling, and then filtering off on the Büchner, using suction, through heavy pads of filter paper to remove finely divided sulphur and mercuric sulphide. The filtrate was now a clear, beautiful greenish-yellow liquid, whose appearance was identical with freshly prepared casein and albumin-free whey. It was made slightly alkaline with NH_4OH, then acid with H_2SO_4, and saturated with ammonium sulphate in substance. After standing for a few hours the precipitate which had formed was filtered off, and an equal volume of absolute alcohol added to the greenish yellow filtrate. After mixing, the alcohol rose to the top with all the pigment. This alcoholic extract was saturated with ammonium sulphate at a temperature of 60-70° C. The concentrated alcoholic layer which formed contained all the pigment. It was removed, concentrated to low volume and cooled in a brine-ice mixture. Some ammonium sulphate crystallized out and was filtered off. The golden-yellow alcoholic filtrate was used for the aldehyde reactions described below.

PREPARATION OF "ACTIVE" ALDEHYDE

According to Garrod, the activity of the acetaldehyde used in his investigations depended upon the production of some decomposition or polymerization product which forms in the aldehyde after standing for some time in the warm sunlight. Fortunately this investigation was conducted during the months from May to October when plenty of warm sunlight was available.

Two samples of acetaldehyde were prepared, one of which was a crude solution distilled from the oxidation of alcohol with potassium dichromate and collected in a receiver cooled in ice water; the other was prepared by diluting some c.p. Merck's acetaldehyde with sufficient absolute alcohol to prevent its rapid volatilization. Both samples were set out in the direct sunlight, in stoppered bottles, for several weeks. Their activity was tested from time to time by adding a few drops to 5 c.c. of dilute urochrome solution (which exhibited no

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absorption bands) and allowing the test tube containing the solution to stand in the hot sunlight or on the steam bath for a few minutes and then examining the solution for absorption bands.

The aldehyde solutions first exhibited signs of "activity" by causing the color changes in the urochrome solutions which are described by Garrod i.e. yellow to orange to brown-red on continued heating. At this stage it was not possible to detect the development of any absorption bands. The aldehyde preparations were allowed to continue in the warm sunlight, however, with a result that they eventually became "active" in their ability to produce the absorption bands described by Garrod.*

ACTION OF "ACTIVE" ALDEHYDE ON UROCHROME

None of the urochrome preparations prepared above exhibited any absorption bands either in neutral solution or after acidifying with HCl. The solutions were also non-fluorescent. On adding about 10 per cent of either of the aldehyde preparations to any of the alcoholic urochrome solutions and allowing the mixture to stand in the warm sunlight or on the steam bath, the light yellow color of the solutions changed to a golden and finally to a deep brown-red color. When examined in the spectrometer at the proper dilution the golden yellow solutions exhibited a beautiful absorption band in an identical position with the normal "acid" urobilin band. On making the solution alkaline with NH_4OH the color of the solution became much fainter and the band disappeared. On adding a few drops of alcoholic zinc chloride solution, a beautiful green fluorescence appeared and the absorption band reappeared, considerably broader and slightly shifted toward the red end of the spectrum. On dilution with alcohol these solutions showed the characteristic pink color of a similar urobilin solution without, however, destroying the green fluorescence. After allowing the action of the aldehyde to continue until the solutions had become red colored, the solutions showed a clear second band in the blue as described by Garrod in connection with his study of "active" aldehyde on urochrome.

These spectroscopic observations were made with an ordinary spectrometer of narrow dispersion using an 80 candle power Mazda lamp for the source of light. The lenses and prism were crown glass.

*Note:—Aldehyde solutions retain their "activity" for a long time. The sample prepared from Merck's c.p. acetaldehyde was still very active after it had stood in the author's laboratory for twelve months.

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The absorption bands were so clear and characteristic that they were photographed. The photographs were made on Cramer's spectrum plates using an Adam Hilger spectrocopic [1] with photographic attachment. They are shown in the accompanying plates. Crown glass lenses and prism were used. An 80 candle power Mazda lamp was used for the source of light, it being arranged in a special box so that only the spectrometer slit and the cell containing the solution were illuminated. A photograph of an electric spark in hydrogen atmosphere was made on each plate so that a few solar lines could be had for comparison, especially the F. line. It was not possible to standardize the concentration of the pigments in the solutions to be photographed. It was attempted, however, to use solutions of such concentration that the absorption bands to be photographed were all clear and as distinct as possible when viewed in 10 mm. layer by the observation spectrometer of narrow dispersion.

Where photographs of the normal urobilin bands were made for comparison the urobilin solutions were prepared as follows: A volume of normal urine whose urobilinogen had been transformed by allowing the urine to stand exposed to the air in the presence of HCl, was shaken gently in a separatory funnel with an equal volume of ether. The ether extract was separated, evaporated to dryness, and the residue dissolved in a few cubic centimeters of alcohol. The alcoholic solution exhibited the characteristic urobilin band and other urobilin properties.

Plate 1, Figures II and III show respectively the normal (acid) urobilin band and the band produced by "active" aldehyde on alcoholic urochrome solutions. Plate 2, Figures II and III show the same bands after being treated with ammonia and zinc chloride. As stated above these solutions showed the beautiful green fluorescence characteristic of "urobilin zinc" solutions.

Plate 3, Figure II again shows the result of "active" aldehyde on urochrome solutions. Figures III and IV of the same plate are two different exposures of the same solution after the reaction of the aldehyde had been allowed to continue until the second band had developed. This is plainly visible in the two photographs.

These photographs very strikingly confirm the observations of Garrod. One can only suppose that the failure of Dombrowski and Holweg to confirm the action of aldehyde on urochrome, was due

1. The authors are indebted to Dr. O. M. Stewart, chairman of the Physics Department, University of Missouri, for the use of this instrument and assistance in making the photographs.

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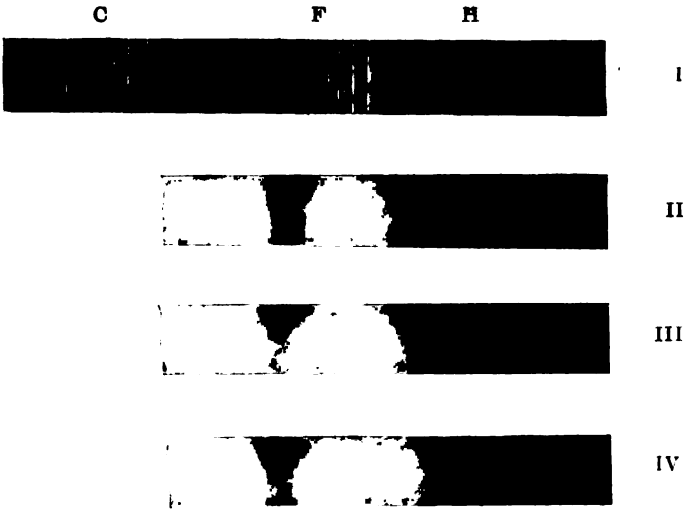

Plate No. 1

I. Spectrum of electric spark in hydrogen.
II. Absorption band of urobilin in acid solution.
III. Band produced by "active" aldehyde on urochrome.
IV. Band produced by "active" aldehyde on lactochrome.

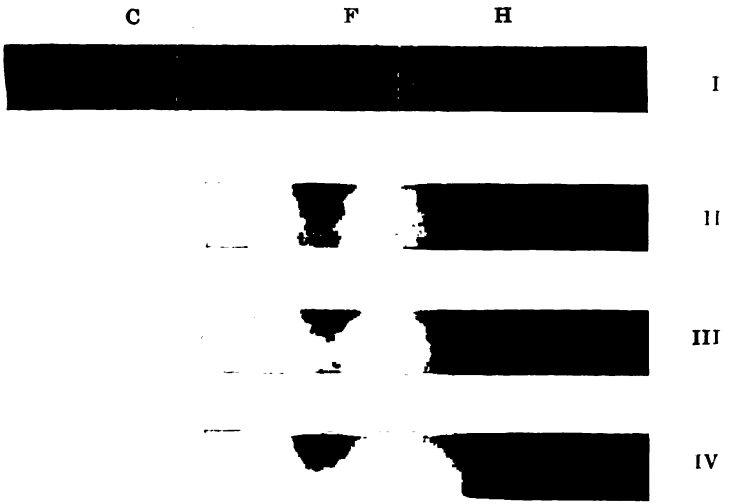

Plate No. 2

I. Spectrum of electric spark in hydrogen.
II. Normal absorption band of "urobilin zinc."
III. Band produced from urochrome by aldehyde after treatment with NH_4OH and $Zn\ Cl_2$.
IV. Band produced from lactochrome by aldehyde after treatment with NH_4OH and $Zn\ Cl_2$.

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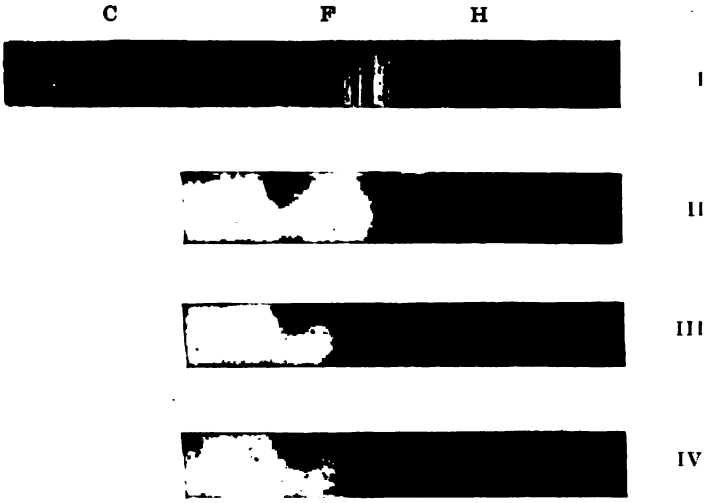

Plate No. 3

I. Hydrogen spectrum.
II. Absorption band produced by aldehyde on urochrome.
III and IV. Two different exposures of urochrome solution in which action of aldehyde had reached the double band stage.

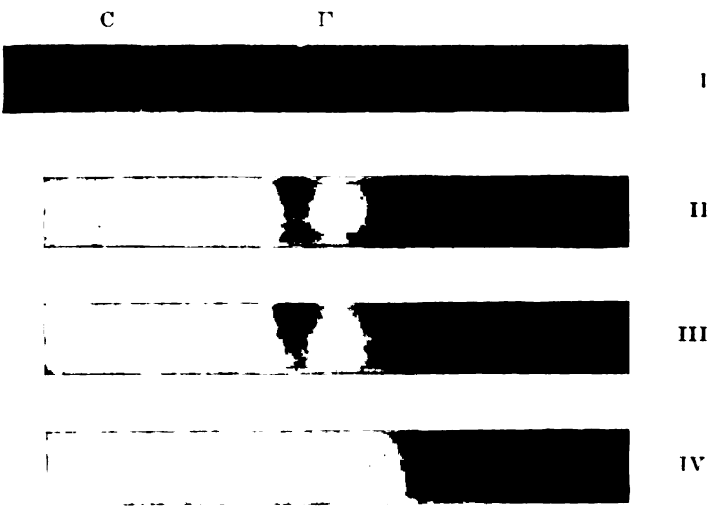

Plate No. 4

I. Hydrogen spectrum.
II, III and IV. Three exposures of a lactochrome solution showing the development of the second band.

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probably to the fact that the aldehyde used by them was not really "active." This seems especially probable in the case of Holweg in the light of observations made at this station in regard to the difficulty in obtaining a truly "active" aldehyde. In the case of Dombrowski's work it is possible that his urochrome preparations were so "pure" that they were in reality decomposed.

THE ACTION OF "ACTIVE" ALDEHYDE ON LACTOCHROME

The alcoholic solution of lactochrome, whose preparation was described above, was spectroscopically inert in both neutral and acid solution. The action of the "active" aldehydes upon the solution, however, was in every respect identical with the action of the aldehydes upon the urochrome solutions. On warming about 10 c.c. of the alcoholic lactochrome solutions to which one cubic centimeter of "active" aldehyde had been added, or allowing the same to stand in the warm bright sunlight the yellow color of the solution deepened to an orange tint and a single beautiful absorption band appeared in an identical position with the normal (acid) urobilin band. A photograph of this band is shown on Plate 1, Figure IV in comparison with the normal urobilin band and the similar band produced by the "active" aldehyde upon the urochrome solutions.

Upon addition of a drop of concentrated NH_4OH to the orange colored solution, the color was changed to a light yellow. The absorption band disappeared entirely. Upon addition of a few drops of alcoholic zinc chloride to this ammoniacal solution a broader band appeared, shifted slightly toward the red end of the spectrum from the original band. This band is shown in Plate 2, Figure IV in comparison with the normal "urobilin zinc" band and the band produced by a similar action upon urochrome solutions. The three bands are seen to be identical. In conjunction with the appearance of this band the solution took on beautiful green fluorescence, and on dilution the color of the solution changed to a dull pink.

Continued action of the "active" aldehyde upon the alcoholic lactochrome solutions caused the color to change to a deep brown-red color with the appearance of a second absorption band.

Plate 4, Figures II, III and IV are three different exposures of the same lactochrome solution showing both absorption bands.

The product which resulted when the transformation of the lactochrome was allowed to proceed only to the one band stage, resembled natural urobilin in that it was almost completely precipitated as reddish colored flakes on saturation with ammonium sulphate. The

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precipitate was readily soluble in ordinary alcohol, and in chloroform, and could not be extracted from the latter solution by water. On evaporation of the chloroform solution into alcohol, the resulting alcoholic solution showed the characteristic urobilin absorption band. A little "active" aldehyde was added to this solution and on continued heating on the steam bath a faint second band appeared without any visible change in the color of the solution. When more aldehyde was added and the solution placed in the strong sunlight the pigment precipitated and would not again dissolve on addition of either water or alcohol.

"Active" aldehyde was found to produce results identical with the above when acting merely on an impure alcoholic extract of casein and albumin-free milk serum which had been saturated with ammonium sulphate. The reaction also proceeded normally with an alcoholic extract of milk whey which had been produced by coagulation of the protein with rennet, and saturation of the filtrate with ammonium sulphate. These results greatly facilitated the spectrographic work. Some of the photographs of the bands produced by aldehyde on lactochrome were made with solutions prepared in this simple fashion.

"Active" aldehyde was found to have a very characteristic action on the greenish-yellow milk serum from which the casein, fat and coaguable proteins had been removed. On standing in the hot bright sunlight or warming on the steam bath, after the addition of about 10 per cent of "active" aldehyde, small volumes of serum which themselves were practically colorless and exhibited no absorption bands either before or after acidifying with HCl took on a light rose color which finally changed to a lavender. At the same time one and finally two characteristic absorption bands appeared. Sometimes the bands were in the normal positions, and again they were considerably shifted toward the red end of the spectrum, Band A extending almost into the yellow. The addition of NH_4OH destroyed the bands and changed the color of the solution to a faint pink.

This result seems to be contradictory to the statement made by Garrod that "active" aldehyde had no action on aqueous solutions of urochrome. An explanation might be found, however, in the fact that the aldehyde preparations used in these studies contained considerable alcohol. The cause of the shifting of the bands does not seem to be so readily explained. It may have been due to the percentage of alcohol present, for it was found that some samples of serum exhibited identical properties on treatment with "active" alde-

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hyde without, however, the shifting of the bands. More alcohol might have been present in these cases. The point to be emphasized is that a purely alcoholic solution of lactochrome was not required for the manifestation of the "activity" of the aldehyde, equally good results having been obtained when only small quantities of alcohol were present.

THE ACTION OF "ACTIVE" ALDEHYDE ON THE PIGMENT OF THE WHEY OF MILK FROM ANIMALS OTHER THAN COWS

It was hoped to study the whey pigment from milk other than cows' milk, extending the study to include a number of other animals. Unfortunately, however, it was possible to make a thorough study of only sheep's milk. In addition one sample of human milk was examined.

The striking characteristic of all the sheep's milk examined was the unusually high color of the whey. Some figures will be given later in connection with some colorimetric studies of milk whey showing how much higher colored the whey of sheep's milk is than the whey of cows' milk. (See Table I.)

The pigment of the sheep's milk whey was found to be identical in every respect with the lactochrome of cows' milk. "Active" aldehyde acting on alcoholic extracts of the ammonium sulphate saturated whey, produced the urobilin-like pigment and its accompanying properties in even more striking manner than when acting on similar solutions from cows' milk. The development of the second band on continued action of the aldehyde was especially pronounced. The solutions were spectroscopically inert before being treated with the active aldehyde.

In the case of the sample of human milk, the well known difficulty of obtaining an acid coagulation of the casein interfered with the observation of the color of the serum. An alcohol soluble yellow pigment giving the reaction with "active" aldehyde was obtained, however, by the following method: A pinch of NaCl and a little $(NH_4)_2SO_4$ was added to about 350 cc. of human milk and the milk brought to a boil after acidifying with acetic acid. The precipitate was filtered off and washed with a little 95 per cent alcohol and then extracted until colorless with ether and hot 95 per cent alcohol. The ether-alcohol extract was evaporated to dryness and the residue freed from fat with ether. An orange-yellow ether insoluble residue was left which readily dissolved in ordinary alcohol. This solution gave the properties of urobilin after treatment with "active"

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aldehyde, turning to a deep red in the warm sunlight and showing a beautiful absorption band. $ZnCl_2$ and NH_4OH caused the usual green fluorescence, the solution at the same time showing the absorption band of urobilin zinc.

Only one conclusion is possible from these results, namely, that sheep's milk and human milk are characterized by the same whey pigment found in cows' milk.

ACTION OF "ACTIVE" ALDEHYDE ON UROBILIN

A few observations were made in connection with these studies on the action of the aldehyde on solutions of natural urobilin. An alcoholic solution of urobilin was prepared by acidifying 100 cc. of fresh urine with HCl, letting stand in the sunlight to transform urobilinogen to urobilin, and extracting with about 50 cc. of chloroform. The chloroform extract was filtered and the filtrate evaporated to dryness at a low temperature. The red colored residue was dissolved in a few cc. of 96 per cent alcohol.

The golden-yellow solution which resulted showed a beautiful absorption band. On adding a little "active" aldehyde to this solution it at once showed a beautiful green fluorescence. After warming for some time on the steam bath the solution when examined in the spectroscope showed a broad band which appeared identical with the ordinary band of "urobilin zinc:" The solution, however, was acid to litmus paper. The appearance of the band differed from the normal band of "urobilin zinc" in that it had the appearance of the normal acid urobilin band laid upon a broader but fainter one. Continued heating caused the wide band to become uniformly intense. No second band appeared and no color change was exhibited other than the production of the green fluorescence already noted. After a time the band disappeared entirely.

These results studied in connection with the photographic plates make it clear that some substance other than urobilin is the cause of the characteristic reactions which urochrome and lactochrome give with "active" aldehyde. The reaction which natural urobilin gave with the aldehyde in the main confirmed the results obtained by Garrod, who found that aldehyde had no action on preparations of urobilin already showing the urobilin absorption properties.

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THE BROMINE COMPOUND OF UROCHROME AND LACTOCHROME

Following the method of Salomonsen [1] and Mancini,[2] bromine was added a drop at a time to a portion of the crude aqueous solution of urochrome prepared as described above. When it was considered that an excess had been added the solution was set aside in the ice box. After twelve hours a yellow precipitate had come down which became granular on rubbing up with water in a mortar. The compound was not examined very closely, but in general answered to the description given by Salomonsen. It was soluble in glacial acetic acid, only slightly soluble in alcohol and insoluble in cold water.

In a similar way bromine was added to a concentrated aqueous solution of lactochrome which had been prepared from four liters of highly colored skim milk whey in the following manner: The acetic acid whey was boiled and filtered. Concentrated NaOH solution was added to the hot filtrate until another coagulation took place. The reaction was still acid. In this way a perfectly clear, beautiful, deep greenish-yellow solution was obtained. Neutral $Hg(NO_3)_2$ solution was added to the cold solution until no more precipitate came down, and the heavy cream colored precipitate removed by filtration on a Büchner funnel, using suction. The precipitate was washed a number of times by suspending it in distilled water and refiltering. It was finally decomposed with H_2S. The black emulsified solution was heated to boiling and concentrated HCl added a little at a time until the HgS came down. This was filtered off, thoroughly washed with water, resuspended in water and more H_2S passed in. The emulsion was treated as before. The filtrate contained quite a little additional pigment. The combined filtrates of about 800 cc. volume had a golden-greenish-yellow color like that of concentrated milk serum. It was saturated with $(NH_4)_2SO_4$ and the pigment extracted with absolute alcohol in the usual way. Several extractions were required to obtain all the color. The combined extracts of about 800 cc. volume were warmed for some time at almost boiling temperature with a considerable quantity of solid $(NH_4)_2SO_4$. About 300 cc. of water were thus removed. After cooling, the solution was placed in an ice-salt bath for several hours. The $(NH_4)_2SO_4$ which crystallized out was removed by quick filtration. This operation was re-

1. Loc. cit.
2. Loc. cit.

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peated until no more crystallization occurred. The alcohol solution was now treated with an excess of $CaCO_3$ to neutralise free acid, thereby strongly reducing the acid reaction of the solution. It was still acid to litmus paper, however. The solution was now concentrated in vacuum at 50° C. to about 50 cc. volume, and was then treated with bromine.

The immediate result of bromination was the precipitation of a brownish-yellow granular substance. When the addition of bromine caused no more of this substance to come down the solution was set aside in the ice box over night. In the morning the precipitate was filtered off on a hardened filter paper and thoroughly washed with cold water, in which it was not soluble.

The Precipitate: The precipitate, after washing and drying, had a brownish-yellow color and granular appearance. In many respects it closely resembled the bromine compound of urochrome described by Salomonsen and Mancini. It was only slightly soluble in hot water and hot absolute alcohol and then only after long digestion on the steam bath. It was, however, readily soluble in both water and alcohol in the presence of a very little alkali (NaOH, KOH, or NH_4OH) giving yellow to brown solutions. Like bromo-urochrome it was precipitated from its slightly alkaline solutions as a green precipitate by $CuSO_4$, as a red precipitate by $FeCl_3$, and as a faintly yellow precipitate by $AgNO_3$. The silver salt was readily soluble in an excess of NH_4OH and was reprecipitated on addition of HNO_3. The latter precipitate became granular on heating and darkened to a brownish color in the sunlight.

The substance gave a strong flame test for halogen, but did not give up its bromine on heating with strong KOH. When heated with zinc dust, the substance gave a strong pyrrol reaction to a pine splinter moistened with HCl.

Contrary to true bromo-urochrome the substance was not readily soluble in glacial acetic acid or 60 per cent alcohol. It was slightly soluble in the glacial acetic acid on boiling and was precipitated from this solution, after cooling, on addition of ether.

On treating the pale yellow, neutral, or slightly acid alcoholic solution with "active" aldehyde and placing it in the strong hot sunlight, the solution turned to a dark brownish-red and showed the absorption band of urobilin. NH_4OH and $ZnCl_2$ changed the color to a golden-yellow and gave the solution a beautiful green fluorescence, the solution at the same time showing the broad absorption band of "urobilin zinc."

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The Filtrate: The filtrate from the bromine precipitate had a deep red bromine color but it did not give up this color on heating or in fact after evaporation to dryness. Instead it left a heavy red colored residue. This residue was only partly soluble in hot absolute alcohol. The absolute alcohol insoluble portion had a flaky appearance and a chocolate-brown color. It was readily soluble in water or dilute alcohol giving solutions of a dull brownish color. The absolute alcohol soluble portion left a reddish-brown gummy residue on evaporation. Its solutions had a brilliant golden-yellow color.

Both portions of the residue were rich in loosely combined bromine, giving it up in alkaline solution so that on neutralization it was readily thrown down by silver nitrate, the white granular precipitate in both cases rapidly turning purple in the sunlight. Neither of the residues gave a pyrrol reaction on heating with zinc dust.

Alcoholic solutions of the chocolate-brown residue gave a strong reaction with "active" aldehyde, quickly turning to a rose and then to a deep red color in the sunlight and showing the urobilin band and other urobilin properties. Solutions of the gummy residue also gave the urobilin properties on treatment with aldehyde, but not so clearly and with much greater difficulty.

THE PYRROL GROUP IN UROCHROME AND LACTOCHROME

An excess of aqueous copper acetate solution (neutral) was added to about 100 cc. of an alcoholic urochrome solution. A heavy flocculent greenish-brown precipitate came down. It was filtered off on a hardened filter paper and washed thoroughly with water and then with alcohol and ether. The dry precipitate was a greenish powder. On heating with zinc dust the heavy fumes which came off gave a beautiful pyrrol reaction to a pine splinter moistened with HCl.

In regard to a pyrrol group in lactochrome, it can only be said that a compound was obtained from milk serum which very closely resembled the bromine compound of urochrome and which gave a strong pyrrol reaction. It should be stated also that a compound was obtained from the same solution which was readily transformed into the urobilin-like pigment by "active" aldehyde but which did not give any reaction for pyrrol.

The significance of this result is not apparent. It is possible that the pyrrol group in the second compound had in some way or at some time during the isolation been broken down, thereby changing the appearance and some of the properties of the original body. A more detailed discussion of this result will be found below.

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FACTORS INFLUENCING THE COLOR OF MILK WHEY

During the course of the investigations of the milk whey pigment a wide variation in the color of the whey from the milk of different animals was noticed. This was especially evident when the color of the whey from sheep's milk was compared with the color of the whey from cows' milk, and was also often evident when merely observing two samples of freshly prepared whey from the milk of two different cows. In order to establish a better standard of comparison and study the factors that might be influencing the amount of lactochrome in the milk the following procedure was adopted.

Freshly drawn milk was run through a cream separator at a uniform speed and the casein precipitated from the skim milk with the smallest amount of 10 per cent acetic acid necessary to cause a clear coagulation. A portion of the cloudy yellow filtrate was then boiled for a few minutes to bring down the coaguable proteins. After filtering, the yellow serum which resulted was in some instances cloudy. To get a clear solution in these cases the filtrate was neutralized with ammonium hydroxide, warmed and filtered. The filtrate was then always perfectly clear. The yellow serum obtained by either of these methods was then placed in a 10 cm. cell and its color compared with the standard color glasses of the Lovibond tintometer. The colors were readily matched. In some cases a few tenths of red were required.

Using the above procedure, color readings of the whey from the milk from 43 cows and 6 sheep were made. The color of the sheep's milk whey is given to show how very much higher colored it is in many cases than the whey from cows' milk. The figures for sheep's milk are given in Table I.

TABLE I.—COLOR OF WHEY FROM SHEEP'S MILK.

Sample No.	Color, 10 cm. layer	
	Yellow	Red
1.	7.0	0.9
2.	7.0	0.5
3.	15.5	0.5
4.	24.0	1.2
5.	3.5	0.2
6.	4.5	0.5

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The samples of cows' milk represent four breeds and include 4 Ayrshires, 4 Shorthorns, 15 Holsteins and 20 Jerseys, all pure bred dairy animals. Their milk production varied from 4.2 to 47.4 pounds per day. The stage of the lactation period of the animals varied from one to thirteen months, and their ages from three to fifteen years. The results of the colorimetric studies have been arranged according to breed, stage of lactation, age of the animal and volume of milk production, the results being given in Tables 2, 3, 4 and 5.

INFLUENCE OF BREED UPON THE COLOR OF MILK WHEY

The results according to breed are given in Table II. The average tintometer reading for the Ayrshires was found to be 4.78 units of yellow; for the Jerseys, 3.59 units of yellow; for the Holsteins, 2.41 units of yellow; and for the Shorthorns, 2.15 units of yellow.

TABLE II.—INFLUENCE OF BREED.

Breed	Herd Number	Units of yellow	Breed	Herd number	Units of yellow
Ayrshire	305	4.5	Shorthorn	400	2.6
Ayrshire	306	4.0	Shorthorn	403	3.0
Ayrshire	307	5.0	Shorthorn	404	1.6
Ayrshire	301	5.5	Shorthorn	406	1.4
	Average	4.75		Average	2.15
Jersey	57	2.9			
Jersey	23	3.0	Holstein	223	1.5
Jersey	11	2.7	Holstein	217	1.7
Jersey	41	3.1	Holstein	208	2.2
Jersey	53	3.5	Holstein	213	2.2
Jersey	16	3.5	Holstein	209	3.0
Jersey	124	5.0	Holstein	204	3.0
Jersey	10	3.5	Holstein	219	2.2
Jersey	3	3.0	Holstein	222	3.6
Jersey	30	2.7	Holstein	227	2.7
Jersey	54	3.0	Holstein	224	2.6
Jersey	50	3.1	Holstein	220	1.3
Jersey	8	3.3	Holstein	210	2.2
Jersey	2	2.7	Holstein	215	3.0
Jersey	13	3.2	Holstein	226	2.3
Jersey	317	5.3	Holstein	211	2.7
Jersey	27	4.5		Average	2.41
Jersey	22	5.2			
Jersey	59	4.5			
Jersey	14	4.2			
	Average	3.59			

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These colorimetric averages emphasize what was found by mere observation of even the cloudy yellow whey after removal of the casein, namely that the Ayrshire and Jersey milk is characterized by yielding much higher colored whey than Holstein and Shorthorn milk. In some cases it was possible to select the samples of Ayrshire milk from a mixed lot of Ayrshire, Jersey and Holstein milk, on account of its relatively higher colored whey. Only in a few cases was the Jersey whey as high colored as the Ayrshire whey.

These figures showing such a marked difference between the average color of the whey of the milk from the four different breeds, when taken into consideration with the fact that each breed represents widely different conditions of milk production, stage of lactation, etc., would indicate that the color of the milk whey is primarily a breed characteristic. Within narrower limits it also appears to be an individual characteristic.

TABLE III.—INFLUENCE OF STAGE OF LACTATION.

Herd number	Stage of lactation (Months).	Units of yellow.	Herd Number	Stage of lactation (Months).	Units of yellow.
Ayrshire			Jersey		
301	10	5.5	317	13	5.3
305	3	4.5	27	13	4.5
306	3	4.0	41	12	3.1
307	1	5.0	23	12	3.0
			22	12	5.2
			54	11	3.0
Holstein			30	10	2.7
222	10	3.6	57	8	2.9
223	10	1.5	14	7	4.2
209	8	3.0	16	7	3.5
215	8	3.0	3	6	3.5
211	8	2.7	124	5	5.0
224	8	2.6	53	5	3.5
227	7	2.7	10	4	3.5
217	7	1.7	8	3	3.3
226	4	2.3	59	1	4.5
213	4	2.2	2	1	2.7
219	4	2.2			
220	4	1.3			
208	3	2.2	Shorthorns		
204	2	3.0	403	8	3.0
			406	7	1.4
			404	1	1.6

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INFLUENCE OF THE STAGE OF LACTATION UPON THE COLOR OF THE WHEY

The results are arranged in Table III according to the stage of lactation of the cows. The differences here are not very pronounced, and are hardly definite enough to warrant any conclusions. A calculation, however, shows that 78.5 per cent of the cows which had been in milk for less than five months gave a color reading slightly below the average of the breed.

INFLUENCE OF THE AGE OF THE ANIMAL UPON THE COLOR OF THE MILK WHEY

The results are arranged in Table IV according to the age of the animals. An examination of the table indicates that the age of the animal may slightly influence the color of the whey. Nearly all the cows over 7 years of age, especially the Jerseys, gave color readings above the average for their breed. Of the cows under 7 years of age, 70 per cent are below the average. This would indicate that the older the cow the higher the color of the milk whey. Three of the old cows, however, i. e. Nos. 317, 27 and 301 were also well advanced in lactation, which may cause the apparent influence of the age to lose some of its significance.

INFLUENCE OF THE VOLUME OF MILK PRODUCTION ON THE COLOR OF THE MILK WHEY

The results are arranged in Table V according to the volume of the milk produced by the animals.

The interpretation of Table III that fresh cows seem to have less color in their milk whey might seem to indicate that the variations in the color may be merely a dilution effect. If the average milk production and average color reading of the Holstein cows are compared with the average milk production and average whey color of the Jersey cows, the highest color evidently accompanies the lowest milk production. This does not hold good, however, when comparing either the Jerseys or the Holsteins with the Ayrshires. Similarly the relation of high color to low milk production does not hold good when comparing the Jerseys or Holsteins among themselves. For instance Jersey Cow No. 124, with the highest milk production, gave one of the highest colored wheys of the series while Jersey Cow No. 30, with a milk production less than one fourth as great gave the lowest colored

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whey of the Jerseys. The high color of the whey from Cow No. 124 might be explained on the grounds of her age as seen in Table IV. The same explanation, however, could not be offered for the high color of Cow No. 59 whose age was only six years. These examples could easily be multiplied both among the Jerseys and the Holsteins, as a consultation of the table will show.

TABLE IV.—INFLUENCE OF AGE.

Herd number	Age (Years)	Units of yellow	Herd number	Age (Years)	Units of yellow
Ayrshire			Jersey		
301	9	5.5	317	15	5.3
305	5	4.5	124	15	5.0
306	4	4.0	16	13	3.5
307	4	5.0	27	10	4.5
			10	7	3.5
			59	6	4.5
Holstein			53	6	3.5
204	12	3.0	41	6	3.1
209	10	3.0	50	6	3.1
211	7	2.7	54	6	3.0
210	7	2.2	57	6	2.9
213	6	2.2	3	5	3.0
215	5	3.0	8	4	3.3
208	5	2.2	23	4	3.3
217	5	1.7	2	4	2.7
222	4	3.6	22	3	5.2
219	4	2.2	14	3	4.2
223	4	1.5	30	3	2.7
220	4	1.3			
227	3	2.7			
224	3	2.6	Shorthorn		
226	3	2.3	403	10	3.0
			404	4	1.6
			406	3	1.4

It must be concluded, then, that the differences in color of the whey among different cows, while perhaps influenced in some cases by the volume of the milk, is not primarily a dilution effect. If any interpretation at all can be put upon the figures given in the tables it seems that the variation in the color is primarily a breed characteristic, as stated before, with the milk yield, age of the animal and possibly the period of lactation as minor influencing factors.

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TABLE V.—INFLUENCE OF THE VOLUME OF MILK PRODUCTION.

Herd No.	Milk produced	Color of whey	Herd No.	Milk produced	Color of whey
Holstein			Jerseys		
210	47.4	2.2	10	28.0	3.5
204	46.0	3.0	16	27.2	3.5
211	41.6	2.7	3	23.6	3.0
213	40.8	2.2	54	19.0	3.0
209	38.4	3.0	53	18.4	3.5
219	34.2	2.2	41	15.0	3.1
208	30.0	2.2	14	14.2	4.2
223	29.6	1.5	12	13.6	2.7
217	28.8	1.7	317	13.4	5.3
226	27.2	2.3	50	13.0	3.1
220	23.0	1.3	8	12.4	3.3
227	20.8	2.7	27	12.4	4.5
215	14.0	3.0	57	10.8	2.9
224	12.0	2.6	11	10.0	2.7
222	8.0	3.6	30	8.4	2.7
			22	8.0	5.2
			13	4.2	3.2
	Av. 29.4	2.41			
Shorthorn				Av. 16.8	3.59
404	27.2	1.6			
403	26.0	3.0	Ayrshire		
400	24.0	2.6	305	32.0	4.5
406	11.2	1.4	307	30.8	5.0
			306	25.0	4.0
			301	19.4	5.5
	Av. 22.1	2.15			
Jersey				Av. 26.8	4.75
124	36.0	5.0			
59	33.4	4.5			

INFLUENCE OF FEED UPON THE COLOR OF MILK WHEY

In addition to the foregoing studies it was considered advisable to study the influence of the food of the cow upon the color of the whey. This would seem to be especially important in view of the fact that in a contemporaneous [1] investigation the food was found to be the important factor in the pigmentation of the milk fat.

The color of the milk whey was observed with two different cows in two different succeeding periods in which the food had been varied primarily for the purpose of studying the effect upon the color of the milk fat. The results are given in Table VI.

1. Missouri Agricultural Experiment Station Research Bulletin No. 10 (1914); Jour. Biol. Chem. 17, p. 191 (1914).

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TABLE VI.—INFLUENCE OF FEED UPON THE COLOR OF MILK WHEY.

Cow No.	Period	Feed	Color of Fat	Color of Whey
			1 inch layer Yellow	10 cm. layer Yellow
57	1	Bleached clover hay and white corn....	8.0	3.5
57	2	Green alfalfa hay and yellow corn.....	45.0	4.5
			Yellow	Yellow
301	1	Green alfalfa hay, corn silage and grain.	32.0	5.5
301	2	Bleached timothy hay and white corn...	1.3	3.0

The results of these two cases might seem to indicate that some feeds influence the color of the whey as well as the color of the milk fat. This is emphasized in the case of Cow No. 301 because the milk production in the two periods is known to have been the same. If there is an influence upon the color of the whey it certainly is not nearly so pronounced as it is upon the color of the milk fat. Besides, this apparent result is of no value whatever in explaining the wide difference between the color of the whey of the different breeds as shown in Table II for when those readings were taken all the cows were on the same ration consisting of alfalfa hay, corn silage and a grain mixture of corn, bran and oil meal.

It is to be concluded, then, that the feed has little or no influence upon the color of the milk whey. Certainly slight individual variations in the color of the whey are to be expected from time to time and it was probably merely accidental that the color of the whey was somewhat lower in each case in the period when the milk fat was very low colored, for there was a considerable lapse of time in both cases between periods 1 and 2. This was 12 days for Cow No. 57 and 8 weeks for Cow No. 301. This question was not considered of sufficient importance to pursue further.

DISCUSSION OF RESULTS

A study of the results of the chemical investigation of the whey pigment leaves no doubt of its very close relationship to urochrome, the specific pigment of normal urine. The general characteristics of the two pigments are identical. Both pigments are precipitated by the

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same reagents, and in common they are not precipitated to any extent on saturation of their aqueous solutions with ammonium sulphate, but can be extracted from the latter solution by alcohol. Again, both pigments show the same solubilities, and their solutions are spectroscopically inert.

Solutions of both pigments give the same striking reaction with "active" acetaldehyde, being transformed thereby into a pigment whose spectroscopic and other properties are identical with urobilin. This is clearly shown in the spectrophotographic plates 1, 2, 3, and 4.

In addition to the above identical properties of the pigments a bromine compound was obtained from a concentrated solution of the whey pigment, whose properties were identical in almost every respect with the bromine compound of urochrome which has recently been made by Salomonsen and Mancini. Solutions of the bromine compound showed the very interesting property of still reacting with the "active" aldehyde and yielding a solution showing the properties of urobilin, a result which might lead to the supposition that this reaction was due to some group or groups in the urochrome molecule or to some accompanying substance, rather than to the pigment itself.

It is clear, however, that the entire whey pigment was not recovered as the bromine compound which so closely resembled the bromine compound of urochrome. Two other bromine compounds were obtained whose properties were in many respects unlike the urochrome-like compound, being readily soluble in water and alcohol and giving no pyrrol reaction. One of the compounds was very reactive with "active" aldehyde, the other less so.

The most reasonable explanation of this result would seem to rest on the supposition that a part of the whey pigment had undergone decomposition at some time previous to bromination. There are several grounds for this supposition. In the first place urochrome is known to readily decompose, especially with mineral acids, which were undoubtedly present (as HCl) in the isolation under discussion. Again it is to be remembered that before any attempt at isolation, the whey pigment always showed the same uniform properties, leaving no doubt that the whey owes its color to but one pigment. Again the secondary substances which were obtained were also bromine compounds, altho clearly of a different character, and one of them i. e., the absolute alcohol insoluble one, was still very strongly reactive with aldehyde. Finally, the fact that the bromination of the whey pigment did not give a quantitative yield of the urochrome-like compound is not an unusual result in reactions of this kind. A complete trans-

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formation of the pigment into its bromine compound could hardly be expected. Certainly Salomonsen and Mancini give no indication that their yield of bromo-urochrome was a quantitative one.

The general conclusion which might therefore be accepted is that the whey owes its color to the same specific pigment as normal urine; that this pigment is readily transformed by "active" aldehyde into a pigment with marked resemblance to urobilin; and that the pigment is readily decomposed and at some stage in the decomposition gives two or possibly three different bromine compounds, all of which may still be transformed into the urobilin pigment by "active" aldehyde.

Although the above interpretation of the results seem the most acceptable, it is admitted that an explanation of the phenomenon of the transformation into urobilin by aldehyde may rest on other grounds. Reference has already been made to this, namely that this property may have been due to some group or groups in the urochrome molecule or to some foreign substance accompanying the urochrome. This view would readily explain why the bromine compounds were still reactive with the aldehyde. Moreover the conclusion would still be justified that the whey pigment is very closely related to, if not identical with urochrome, and that the several additional bromine compounds obtained were due to some previous decomposition of the whey pigment.

The exact nature of the accompanying foreign substance which could be transformed into urobilin by aldehyde is not brought out by the present investigation. It has been definitely shown, however, that if this foreign substance exists, it is present as such in the milk whey and follows the pigment of the whey through its entire isolation by the methods used in this investigation. According to Dombrowski,[1] Garrod's preparations of urochrome which gave the reaction with aldehyde were contaminated with oxy and antoxy proteic acids which, according to the same author, are different steps in the breaking down of the protein molecule which ends in the pigment urochrome. It will be recalled that Dombrowski's preparations of urochrome which were presumably free from these proteic acids, did not give the reaction with a preparation of acetaldehyde which he considered to be "active." Dombrowski believed that Garrod's results were due to some chromogen accompanying the urochrome. If this view be correct, it must therefore be concluded that the same chromogen accompanies the urochrome in both the milk whey and urine.

The results of this investigation are of some importance from a physiological standpoint as they furnish additional proof that uro-

(1). Loc. cit.

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chrome probably originates from the blood rather than from the bile. No conclusions can be drawn from this work, however, as to whether urochrome is a decomposition product of protein or of blood pigment. The constant presence of urochrome in the whey of cows' milk is in itself an interesting physiological phenomenon, especially since it was also found in some cases in relatively very large amounts in the whey of sheep's milk and in small amount even in human milk.

Some interesting observations were made during the course of the investigation relative to some factors influencing the amount of urochrome in the whey, with the result that the breed of the cow was found to be the most important factor although some difference was also found among individuals of the same breed. This difference in the pigmentation of the whey from the milk of cows of different breeds may be of considerable importance in giving to milk its characteristic yellow color. The so-called blue color of milk, especially skim milk, which is so commonly referred to among milk consumers is undoubtedly due to the absence of lactochrome in the whey. On the other hand some samples of milk never seem to lose their yellow appearance even after much skimming. This is especially true of Jersey milk and is usually associated in the popular mind with the "richness" of the milk. Numerous observations during the course of this investigation have shown that this phenomenon is due to the relatively high lactochrome content of Jersey milk rather than to the high fat content.

SUMMARY

1. Lactochrome, the yellow pigment of milk whey, is very closely related in chemical and physical properties to urochrome, the specific yellow pigment of normal urine, and is very probably identical with it.

2. Alcoholic solutions of lactochrome, or aqueous solutions containing a little alcohol, on treatment with "active" acetaldehyde and heat, are transformed into solutions whose spectroscopic and other properties are practically identical with those of urobilin. On continued action of the aldehyde a secondary pigment is formed with still different spectroscopic properties. In these two properties lactochrome is identical with similar solutions of urochrome.

3. A concentrated aqueous solution yields a yellow granular compound on bromination which gives a strong reaction for pyrrol, and in almost all of its other properties is identical with a similar compound obtained on bromination of a concentrated aqueous solution of urochrome.

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4. The presence of lactochrome was found to be characteristic of the milk of all breeds of cows tested, i. e., Ayrshire, Jersey, Holstein, and Shorthorn. The amount of lactochrome appears to be largely a breed characteristic, with the Ayrshire and Jersey breeds ranking considerably above the Holstein and Shorthorn.

5. The presence of comparatively large amounts of lactochrome in the milk of some animals is of considerable importance in imparting to milk its characteristic yellow color.

6. Lactochrome was found in sheep's milk, often in much larger quantities than in cows' milk, and was also found in traces in human milk.

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AGRICULTURAL EXPERIMENT STATION

SAP STUDIES WITH HORTICULTURAL PLANTS

COLUMBIA, MISSOURI
May, 1914

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SAP STUDIES WITH HORTICULTURAL PLANTS

W. H. CHANDLER.

SUMMARY.

A very small proportion of the osmotic strength of leaf and cortex sap as measured by the lowering of the freezing point is produced by electrolytes, except in the leaves of succulent plants. It is also true that generally more than half of the osmotic strength of leaf and cortex sap is produced by neither sugars nor electrolytes. Pages 495 to 503.

The molecular weight of the sap solute varies for different tissues, but for any given tissue it does not vary greatly except in early summer during the period of rapid growth when it seems to be somewhat smaller than at other times. The molecular weight of the sap solute of twigs from peach trees that have been forced into vigorous growth by pruning seems to be smaller during most of the summer than that of the cortex sap of peach twigs from trees not so pruned. Pages 503 to 509.

During the growing period there is a fairly constant increase in the molecular concentration of the cortex sap from the roots up through the trunk and large branches to the twigs, exceptions to this, apparently, being most common in the trunk. In late winter this greater molecular concentration of the cortex sap in the upper portions of the tree does not generally prevail. Pages 509 to 512.

The molecular concentration of cortex sap is the smallest during the period of rapid growth. In case of roots, especially, and to a lesser extent in other tissues, the molecular concentration of the cortex sap is not as great during early summer with trees that have been forced into vigorous growth by heavy pruning as with trees not so pruned. Pages 512 to 520.

A molecular concentration as determined by the freezing point seems a fair measure of the condition of nutrition, at least of the roots. Pages 520 to 524.

The molecular concentration of the young leaves near the growing point of peach and apple twigs is not as great as that of the old leaves. Pages 524 to 525.

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The molecular concentration of the leaves of fruit trees is generally considerably greater than that of the fruit except in the case of some ripe fruits like cherries or currants. Where this difference prevails the leaves are able to remove water from the fruit. There seem to be times in the orchard when the air is very dry, and the moisture supply limited when a large foliage may remove the water from the fruit to an injurious extent. Pages 525 to 534.

PURPOSE OF THE STUDIES.

In a study of killing of plant tissue by low temperature [1] it was thought at first that possibly changes in the sap during the winter might help to account for the increased hardiness of mature winter tissue. Accordingly, throughout the winter season and during part of the summer season, freezing point determinations, electrical resistance and molecular weight determinations were made of sap from the cortex of the tissue. It was impossible to make these determinations from the actual sap of buds because it would be impractical to get enough sap. No changes, however, could be found that would give any information as to the nature of the change by which plants acquire maturity (the greater resistance to cold to be observed in winter). These data then, as well as those from other sap studies, are published in this bulletin for any value they may have in determining the nature of the sap solute of horticultural plants.

METHOD OF SECURING SAP.

The determinations were all made from expressed sap. Few determinations were made from sap of sap wood since this could not be secured except at special times in late winter by tapping the trees. Sap could not be expressed by grinding the sap wood and subjecting it to any pressure that was available. In case of nearly all of the trees examined, however, cortex sap could readily be secured in this way. If molar concentration comparisons were to be made for varying seasons, it was necessary to secure the tissue, especially during the summer, at the same time in the day. The molar concentration of the sap of leaves and, to some extent, of twigs, is greater in the afternoon, especially on a clear day, than in early morning, even though the superficial moisture has been removed. Of course if there is much dew on the leaves, necessarily the molar concentration indicated will be smaller. The materials to be compared from day to

1. Research Bulletin No. 8, Missouri Agri. Exp. Sta.

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day were therefore gathered about eight o'clock in the morning during summer, and from nine to ten o'clock during winter, or as soon as the dew was fully off the tissue. On cloudy days the tissue was gathered somewhat later, or if it were excessively dry, clear weather it was gathered somewhat earlier. Of course error could not be entirely eliminated in this way, but it was probably reduced to a minimum.

Twigs for determinations in winter were generally gathered only on days when the tissue was thawed. If frozen periods were prolonged, the twigs were gathered and placed with their ends in water until they became apparently fully turgid. In all cases effort was made to use equally turgid tissue.

In securing sap from twigs, the cortex was scraped from the twigs under a low frame covered with a moist cloth, the frame being open on the side next to the operator. This cortex was then finely ground in a food grinder and the sap expressed by use of the apparatus shown in Figure I. The large blocks with the hole in the center were made of dry hard maple that would not split. The small blocks were made of the same material and of the same contour as the hole turned in the large blocks and just enough smaller so two or three ply of eight-ounce duck would go between the surfaces. Before using the first time both blocks were immersed in hot paraffin to make them impervious to the sap. The plant tissue was wrapped in eight- to ten-ounce duck, pressed into the hole in the large block and the small block set against it. These were placed in the press made of pieces of 4″x4″ lumber drawn together by use of two ordinary bench screws, one at each end. There was no difficulty in securing the sap very readily from the ground cortex of practically all twigs, and from the cortex of nearly all tree trunks and large limbs.

Sap from leaves was secured in the same way as that from twigs. In the case of peach, plum and pear leaves, however, it was very difficult to express the sap. It could not be expressed rapidly enough from these to avoid evaporation from the drops at the base of the block sufficient to cause the sap to show a greater molar concentration than it should. Usually it took nearly twenty-four hours to express enough sap from a block full of ground peach leaves for a freezing point determination. Hence with these leaves there was inevitably some error caused by evaporation.

Another source of error in this method is the fact that the first sap expressed in this way does not generally show as great molar concentration as sap coming from the same tissue when it is

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pressed more nearly dry. The following table will give an idea of the error involved.

TABLE I.—FREEZING POINT DEPRESSION OF FIRST SAP EXPRESSED FROM PLANT TISSUE COMPARED WITH THAT OBTAINED BY APPLYING ADDITIONAL PRESSURE TO THE SAME TISSUE.

Variety	Date	First sap expressed	Second sap expressed	Last sap expressed*
Elberta twigs.....	Sept. 5, '08	2.024	2.161	2.552
Elberta twigs.....	Sept. 5, '08	2.213	.....	2.846
Elberta twigs.....	Sept. 5, '08	2.038	.....	3.172
Elberta twigs.....	Sept. 5, '08	1.815	.....	2.210
Elberta leaves.....	Sept. 5, '08	2.258	2.387	2.721
Elberta leaves.....	Sept. 5, '08	1.977	.....	2.600

*Tissue very dry.

This is probably in line with the results of Dixon and Atkins,[1] who found that when the protoplasmic membrane was rendered permeable by use of liquid air the expressed sap showed a greater molecular concentration.

However, the possible error shown in the table was taken into consideration and effort was made to press all tissue as nearly dry as possible, so that the error was probably small except in the case of peach, plum and pear leaves.

For freezing point and electrical resistance determinations the sap was not filtered and was used as soon as possible after being expressed. If it had to be kept for a number of hours, it was kept on ice.

METHOD OF MAKING FREEZING POINT DETERMINATIONS.

The purpose of freezing point determinations was to get an idea of the total molar concentration of sap, making use of the experimentally determined fact that the molecular weight in grams of any nonelectrolyte in 1000 grams of water will lower the freezing point about 1.86° C. (electrolytes on account of dissociation, lowering it to a greater extent). The usual Beckmann freezing point apparatus was

1. Dixon, H. H., and Atkins, W. R. G.—Methods of Extracting Sap from Plant Organs, Sci. Proc. Roy., Dublin Soc. N. Ser. Vol. 13, No. 28, (1913), pp. 422-433, and Cryoscopic and Conductivity Measurements in Some Vegetable Saps, same Vol. 13, No. 29, pp. 434-440.

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used. All of the recognized precautions for accuracy in the use of this apparatus were taken with the exception that not in all cases was an effort made to read the thermometer to the third decimal place. Neither were extended readings and curves made to determine the actual freezing point of the sap, eliminating the necessary error caused by slight supercooling. The errors in getting the sap were no doubt sufficiently large that those last two precautions would not add to the accuracy of the determination. The important precaution of preventing great supercooling by use of a small ice crystal dropped into the sap at approximately its freezing point, was observed, and freezing point determinations of distilled water for comparison were made at the beginning and end of each day's determinations. Where it was possible to secure enough sap, the freezing point determinations were always checked with a new sample. However, even where the ice crystal was taken out of the sap with but slight melting, the same sample could not be used for a second determination as a check because in practically all cases a second freezing point determination of plant sap would show a higher freezing point than the first. This may possibly result from some polymerization of certain sap solutes, at least these same phenomena were not observed when sodium chloride solutions were frozen the second time.

DETERMINING ELECTRICAL RESISTANCE.

The electrical resistance was determined from parts of the same samples of sap from which freezing point determinations were made. The apparatus used for determining the electrical resistance was a Kohlrausch universal bridge. With this apparatus the scale readings under the bridge wire give the ratio of the bridge arms, and so the resistance of the solutions is obtained by simply multiplying the known resistance by the observed ratio. In order to be able to make use of samples of sap not larger than two cubic centimeters, the electrodes were made somewhat small, about one centimeter square. They were so adjusted that there was no great variation in the cell constant from time to time, yet resistance determinations for one-fiftieth normal potassium chloride solutions were made with each day's running. The determinations were all made at a temperature of 25° C. It was possible to keep the temperature constant by the use of a well insulated germinating oven. The cell and mercury connection cups were placed inside the oven with the wires running through the opening for the thermometer to the bridge on the outside of the oven. In the oven the cell was placed in a pan of water in

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which a thermometer bulb was kept. The oven was surrounded by a water jacket and insulated with wool felt so temperature changes took place very slowly. Frequent thermometer readings were made, however, and since the room temperature was sometimes below 25° C. it was occasionally necessary to light a Bunsen burner under the oven. In the tables the readings have been reduced to specific conductivity by comparing them with resistance determinations of one-fiftieth normal potassium chloride. The constant of the cell used was determined by dividing the known specific conductivity of one-fiftieth normal potassium chloride (2.768×10^{-3} reciprocal ohms) by the reading obtained with the cell used for one-fiftieth normal potassium chloride. The specific conductivity of the saps was calculated by multiplying the conductivity (reciprocal of resistance) found with the cell used by the figure obtained for the cell constant. Another column in the table also gives the percentage of a normal solution of potassium chloride that would have the same resistance. These were determined by making use of the table on page 744 of Vol. 3 of Landolt, Börnstein und Meyerhoffer, Physiklaische-Chemische Tabellen. The figures for concentration given there, after correcting for the difference in temperature, were used to make the curve by which the specific conductivity for concentrations between those given in the table could be secured.

The Department of Agricultural Chemistry has supplied reducing sugar analyses from samples of this sap. Table 3 shows the percentage of reducing sugar and also the percentage of a molar solution (the molecular weight in grams in a liter of water) represented by the sugar of the sap. The percentage of a molar solution represented by all of the materials in the sap was determined by dividing the determined freezing points of the sap by 1.86.

The following table then gives the percentage of molar solution of all of the sap solutes as determined by the lowering of the freezing point; the percentage of molar solution as shown by the reducing sugar determination; the percentage of a normal potassium chloride solution that will have the same electrical resistance, and the percentage of a molar solution that is neither reducing sugar nor an electrolyte.

In calculating the part of the total osmotic strength of the sap caused by electrolytes, the figures for KCl equivalents are multiplied by 1.90 since it was assumed at the dilutions used the percentage of disassociation for KCl would not be far enough from ninety to affect the accuracy of these results.

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TABLE 2.—SAP FROM CAMBIUM AND CORTEX OF ELBERTA PEACH AND JONATHAN APPLE TWIGS

Material	Date	Percentage dextrose in sap	Molar strength of dextrose	Specific conductivity of sap	Normality of KCl solution of same specific conductivity	Freezing point depression of entire sap	Molar strength of sap	Molar strength of sap minus molar strengths of sugar and electrolytes
Elberta peach	January, 1908	5.675*	.334	.00905	.068	1.902°	1.023	.560
	February, 1908	4.939*	.286	.00959	.073	1.841°	.989	.565
	March, 1908	3.044	.174	.00868	.066	1.758°	.945	.646
	November 7, 1908	4.29	.249	.00788	.059	2.350°	1.263	.902
	November, 1908	5.41	.318	.01032	.079	1.765°	.947	.479
	December, 1908	6.420	.318	.00905	.068	1.69°*	.910	.463
	March 30, 1909°	3.385	.195	.00868	.066	1.845°	.991	.671
	May 1, 1909	2.608*	.149	.00718	.054	1.055°	.567	.315
Jonathan apple	January, 1909	5.073*	.296	.01041	.079	1.902°	1.023	.577
	November, 1909	3.604	.205	.01152	.088	1.924°	1.034	.662
	December, 1909	7.891*	.476	.01152	.088	2.016°	1.083	.440

*NOTE—Averages are given for the month (unless the day of the month is given) for the percentage of dextrose and for the freezing point depressions except in thoses cases followed by * in which only one determination was made. The specific conductivity is not an average but the result of a single determination made some time during the month.

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One slight error should be mentioned. Both the column for sugar determination and that for freezing point determination are given in the terms of a molar solution; that is, the percentage of a gram-molecule of the material in 1000 grams of water, while the column for the results of electrical resistance determination is given in the decimal of a normal solution. In this case there would be a gram-molecule in a liter of the solution; that is, less than a liter of water. A normal solution of a monovalent substance like potassium chloride therefore represents a slightly greater concentration than a molar solution. However, since the percentages of normal solutions in this column are so very small, and since the potassium chloride molecule is small, the difference is far within the range of error in these determinations. It will be seen that in all cases nearly two-thirds of the total molecular concentration of the sap of the tissue shown in this table, (twig cortex of Jonathan apple and Elberta peach in different months during the winter season) is neither sugar nor electrolytes.

When these determinations were made it was thought that the twigs probably contained only reducing sugars. However, in March,

TABLE III.—REDUCING AND NON-REDUCING SUGARS.

Date	March 14, 1913	March 4, 1913	April 4, 1913
Material	Jonathan apple twig sap	Elberta peach twig sap	Elberta peach twig sap
Percentage dextrose before inversion	2.749	2.400	2.093
Molar strength of dextrose	.157	.138	.119
Total percentage dextrose after inversion	7.250	6.709	4.648
Molar strength of invert sugar	.131	.125	.073
Specific conductivity	.01041	.00924	.0102
Normality of KCl solution having same specific conductivity	.080	.070	.078
Freezing point depression of entire sap	1.835	1.735	1.805
Molar strength of sap	.987	.933	.970
Molar strength of sap minus molar strengths of sugars and electrolytes	.547	.537	.630

Note: Depressions were made some time during the same month as the rest of the determinations except the one for March 14, 1913, which was made two months earlier in the season.

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1913, determinations of both reducing sugar and invert sugar were made from Jonathan apple and Elberta peach twig cortex sap, and it was found that in these cases there was as much invert sugar as reducing sugar in the sap, or even more.

TABLE IV.—FREEZING POINT DETERMINATIONS AND CONDUCTIVITIES OF PLANT SAPS.

Date	Material	Freezing point depression	Specific conductivity	Per cent normal KCl solution with same specific conductivity	Fraction of molar solution as shown by freezing point depression	Per cent of total osmotic strength due to electrolytes
	Succulent leaves					
9–21–11	Bean	.985°C	.01313	.100	.530	35.8%
9–21–11	Swiss chard	.810	.01746	.137	.436	59.6
9–21–11	Egg plant	.775	.01839	.142	.417	64.7
9–21–11	Tomato	.680	.01631	.127	.366	65.9
9–21–11	Geranium	.705	.01283	.093	.379	46.7
9–21–11	Sweet potato	.965	.01795	.141	.519	51.6
9–21–11	Morning glory	.920	.01679	.131	.495	50.3
9–21–11	Hollyhock	1.130	.02575	.201	.608	62.8
11– 8–08	Strawberry	2.255	.01748	.137	1.212	21.5
	Jonathan Apple					
7–26–10	Leaves	2.275	.01104	.084	1.223	13.0
8–26–10	Leaves	2.010	.01320	.100	1.081	17.6
7–26–10	Twigs	1.700	.00862	.065	.914	13.5
8–26–10	Twigs	1.505	.00957	.073	.809	17.2
7–26–10	Roots	1.015	.00679	.051	.546	17.7
11–27–10	Roots	1.415	.01268	.097	.761	24.2
	Apple tissue					
6–14–09	Large roots	.907	.00681	.051	.488	19.9
6–14–09	Small roots	.940	.00791	.060	.505	22.6
6–14–09	Trunk	1.092	.00773	.058	.587	18.8
6–14–09	Secondary branches.	1.134	.00662	.049	.610	15.3
	Elberta peach					
6–19–09	Root	.730	.00312	.023	.392	11.1
7–15–10	Root	.960	.00265	.019	.516	7.0
7–15–10	Twig	1.345	.00718	.054	.723	14.2
7–29–10	Twig	1.385	.00632	.047	.745	12.1
7–30–10	Leaf	2.170	.01145	.088	1.166	14.3
8–11–10	Twig	1.725	.00778	.058	.927	11.9
8–12–10	Leaf	2.875	.01336	.103	.155	12.6
8–18–08	Ripe fruit	1.644	.00568	.042	.884	9.0
9– 3–08	Twig	2.031	.01127	.086	1.090	15.0
9– 3–08	Twig (another tree)	1.977	.00884	.067	1.060	12.0
10– 7–08	Twig	2.208	.00868	.066	1.182	10.5
11– 4–08	Leaf	2.365	.01224	.094	1.271	14.0
11– 4–08	Leaf (another tree)	2.325	.01063	.081	1.250	12.3
	Old Mixon Peach					
11– 4–09	Root	1.398	.00539	.040	.752	10.1
11–20–08	Root	1.423	.00544	.041	.765	10.2
11– 4–08	Leaf	3.560	.01321	.102	1.910	10.1
10–30–09	Twig	2.018	.00864	.065	1.090	11.3
11–19–09	Twig	1.870	.00902	.068	1.010	12.7

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In determining the molecular concentration for the invert sugar, allowance is made for the fact that in inverting the sugar, one molecule is changed into two by the taking up of eighteen grams of water.

From the table it will be seen that, even making allowance for invert sugar, there is still about two-thirds of the total molar concentration of the sap that is neither reducing sugar, invert sugar nor electrolytes.

Sugar determinations were not made from leaves nor from summer tissue. Freezing point and conductivity determinations were made for a number of plants. The following table gives these results and the approximate percentage of the total osmotic strength that is due to electrolytes:

It will be seen that in the case of hollyhock, morning glory, egg plant, tomato, sweet potato, beans, and other succulent plants a very large part of the osmotic strength of the sap is produced by electrolytes. When it is considered (Table 5) that the electrical resistance of plant sap increases with dilution much more slowly than the resistance of solutions of potassium chloride, it will be evident that a rather large percentage of the molecules in the saps of these succulent plants must contain a mineral radical.

In case of the peach, apparently the electrolytes constitute a larger percentage of the total sap solute of the leaves than of the twigs, and the smallest percentage of the total solute of the root cortex sap, and of the first sap. In case of apples, however, the proportion of electrolytes to nonelectrolytes seems to be largest in the roots. Leaf sap in all cases, except the one Jonathan apple root sample, has the greatest conductivity, but it is also the most concentrated.

It should be said, however, that these figures really do not give an adequate estimate as to the amount of electrolytes in solution, for the reason that the electrolytes in undiluted saps have a low specific conductivity. The following table illustrates this fact:

In the first two columns is given the specific conductivity of the undiluted sap and the decimal of a normal potassium chloride solution that would have the same specific conductivity. In the next column is given the specific conductivity of the sap diluted one-half, i. e. an equal volume of water added, the conductivity then being multiplied by two. It will be seen that there is always a marked increase in the specific conductivity. In the next column is given twice the decimal of a normal solution of potassium chloride that would have a specific conductivity equal to that of the sap diluted one-half. If the electrolytes in the undiluted sap increased in specific conductivity only

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TABLE V.—SHOWING EFFECT OF DILUTION ON ELECTRICAL CONDUCTIVITY OF PLANT SAPS AS COMPARED WITH THE EFFECT ON A PURE MINERAL SOLUTION.

Date	Tissue from which sap was expressed	Undiluted		Diluted to ½ H_2O		Diluted to ¾ H_2O	
		Specific conductivity	KCl equivalent	Specific conductivity x 2	KCl equivalent	Specific conductivity x 4	KCl equivalent
7–26–10	Jonathan apple leaf	.01104	.085 N	.01632	.124 N	.02105	.157 N
8–26–10	Jonathan apple leaf	.01320	.102 N	.02022	.155 N	.02422	.181 N
1–19–10	Jonathan apple twig	.01041	.080 N	.01293	.097 N	.01593	.118 N
8–26–10	Jonathan apple twig	.00957	.073 N	.01305	.098 N	.01475	.109 N
7–26–10	Jonathan apple root	.00679	.051 N	.00825	.061 N	.01008	.074 N
11–27–10	Jonathan apple root	.01268	.098 N	.01574	.119 N	.01788	.133 N
7–30–10	Elberta peach leaf	.01145	.088 N	.01574	.119 N	.01896	.141 N
8–12–10	Elberta peach leaf	.01336	.103 N	.02018	.155 N	.02502	.187 N
7–29–10	Elberta peach twig	.00632	.047 N	.00827	.061 N	.01000	.073 N
8–11–10	Elberta peach twig	.00778	.059 N	.01052	.078 N	.01244	.091 N
6–19–09	Elberta peach root	.00312	.023 N	.00437	.032 N	.00523	.037 N
7–15–10	Elberta peach root	.00265	.019 N	.00359	.026 N	.00440	.031 N
10–30–09	Oldmixon peach twig	.00864	.066 N	.01201	.090 N	.01443	.107 N
11–19–09	Oldmixon peach twig	.00902	.069 N	.01203	.090 N	.01451	.107 N
11– 4–09	Oldmixon peach root	.00539	.040 N	.00727	.054 N	.01024	.074 N
11–20–08	Oldmixon peach root	.00544	.0405N	.00740	.055 N	.00922	.068 N
9–21–11	Stringless green bean	.01313	.102 N	.01785	.136 N	.02183	.163 N
9–21–11	Egg plant leaf	.01837	.142 N	.02130	.164 N	.02376	.178 N
9–21–11	Tomato leaf	.01631	.127 N	.01926	.148 N	.02227	.1665N
9–25–11	Geranium leaf	.01283	.0995N	.01469	.111 N	.01675	.124 N
9–25–11	Sweet potato leaf	.01795	.141 N	.02440	.189 N	.....	.....
11– 8–11	Strawberry leaf	.01745	.137 N	.02440	.189 N	.03077	.233 N

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as much as potassium chloride increases on account of the dilution, then the figures in the fourth column should be the same as the figures in the second column. It will be seen, however, that uniformly they are considerably larger. This indicates either that the electrolytes in the undiluted sap are not dissociated to anything like as great an extent as the potassium chloride would be in a solution of the same specific conductivity, or what seems more probable, that the viscosity of the sap hinders the movement of the ions. In the next two columns are similar figures for the second dilution of the sap. The first of these contains four times the specific conductivity of the sap diluted to one-fourth, and the other four times the decimal of a normal potassium chloride solution that would have the same specific conductivity. The figures in the last column also should be the same as the figures in column two, if the electrolytes in the plant sap did not increase in equivalent conductivity with dilution more rapidly than would be expected. These figures do not agree with the results of Heald [1] who found the conductivity of the sap and of sap which had been ashed and then diluted to its original volume to be about the same. It would be expected that ashing the sap would increase its conductivity, if the reduced conductivity is due to viscosity of the solvent, since the viscous organic substances would be burned out in ashing.

It will be seen from the preceding tables that the electrolytes play a rather small part in making up the total osmotic pressure of the cells of most plant tissue. It is barely possible that these electrolytes play an even smaller part than is indicated. It seems highly probable that a large percentage of electrolytes are in combination with complex organic molecules. In this case the organic molecules are probably present in the sap any way so all that is added to the osmotic pressure of the cell by the presence of electrolytes is represented by the increase of osmotic strength of the molecules on account of dissociation.

At the beginning of the work reported here it was thought possible that by fertilizing with compounds like potassium salts the osmotic strength of the cell could be increased and that this would increase its resistance to low temperature [2] particularly in case of peach wood and buds. In the work at this station neither the osmotic strength nor the hardiness were increased by the use of potassium fertilizers. From the preceding data it will be seen that a relatively large increase over the

1. Bot. Gazette, Vol. 34, pp. 81-92.
2. Chandler, W. H.—Killing of the tissue of horticultural plants by low temperature. Mo. Agri. Exp. Sta. Research Bul. No. 8.

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approximately twenty-five hundredths of one per cent of potash [1] in peach twigs would increase the osmotic strength so slightly that it would be hardly perceptible. The trees grew in a rather fertile, heavy clay soil with about 23,400 pounds of potassium in the top seven inches of an acre.

MOLECULAR WEIGHT DETERMINATION OF PLANT SAP SOLUTE.

Pursuing further [2] the studies of the nature of the sap solute and changes that may take place through the year, it was thought worth while to secure the molecular weight of sap of twig cortex and the cortex of other parts of the tree during various seasons of the year.

The method of determining the molecular weight was to determine the freezing point of filtered sap, weigh out generally four samples and evaporate to dryness, and figure the molecular weight from these data, by use of the following formula $M=K\frac{w}{dw}$ where w is the number of grams of a substance dissolved in w grams of a solvent; d is the number of degrees that the freezing point of the solution is lower than the freezing point of the solvent, and k is a constant for the solvent. In case of water, k is 1860. The following table gives the result of these determinations. There are given two columns for molecular weights; one shows the largest and the other the smallest molecular weight from each set of determinations of sap from the same tissue. This is intended to give some idea as to the range of error in getting the weights in making the molecular weight determinations. It should be said, however, that all of these samples were run from the same set of freezing point determinations so it gives neither the range of error involved in making freezing point determinations, nor that in the method of collecting the twigs and scraping and expressing the sap. For molecular weight determinations, however, there was little error involved in the method of getting the sap, since, for molecular weights, the freezing point determination was taken after the filtering, there being no intention that these determinations should represent the actual osmotic concentration of the sap. Any evaporation of water involved in getting the sap would then affect equally the freezing point determination and the weighings from which the molecular weights were determined. If the sap stood for any length of time before the freezing point determination was made, there could be polymerization or perhaps other changes that would affect the molecular weight of the solute. However, this was kept in mind and the freezing point de-

1. Van Slyke, L. L., Taylor, O. M., and Andrews, W. H., N. Y. (Geneva) Exp. Sta. Bult. 265, Part III.
2. L. Maquenne. "Sur la pression osmotique dans les graines germes" Compt. rend. 123:898. 1896.

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terminations were taken as soon as possible after the sap was expressed. All of this sap filtered with great difficulty so it was impossible to prevent its standing at least several hours in some cases while being filtered.

It will be seen that the variation is rather wide. This is partly due to the considerable range of error in such determinations, and, unquestionably, rather largely to the actual variation in the amount of solute in the various twigs. When this is compared with the sugar analysis, especially that for invert sugar shown in Table II, it will be seen that the range of variation is not wider than is the difference in the percentage of sugar in seasons not far apart.

The molecular weight of the solute of the peach twig cortex does not vary conspicuously through the season. What small differences there are, however, indicate that the molecular weight is greatest during September, October, November, and December, etc., and smallest during May, June, and July. Thus, in the case of Elberta twigs from trees normally pruned, the average molecular weight of the cortex solute was 167.93 for May, 163.00 for June, 173.03 for July, 172.98 for August, 184.33 for September, 180.05 for October, 180.74 for November, 181.23 for January, 172.30 for February, and 172.99 for March. With Oldmixon Cling it was 163.67 for May, 183.58 for November, 163.49 for January, and 166.47 for February. That for Snow in June was 163.67 and in November, 180.98. A large number of determinations are required before one can be justified in concluding positively that the molecular weight of the cortex sap solute is smaller in spring and early summer.

It is also interesting to note that of seven determinations each, from twigs of trees with normal growth, and from trees that had been cut back severely so they made very vigorous growth and continued growth later in summer, the molecular weight was smaller from twigs of cut back trees, the average of the seven determinations for cut back trees being 170.99 and for normal trees, 183.97. Apparently, a similar difference is to be observed in case of leaves from normal as compared with cut back trees. Again, there was hardly a sufficient number of determinations to justify positive conclusions, yet the results are so uniform as to leave little doubt. It will be seen later that the sap density is lower in case of trees making the more vigorous growth as cut back trees do, during the period of rapid growth in early summer. It would seem possible, then, that in some cases, at least, the rapid use of the plant nutrients in growth leaves a larger percentage of materials with small molecules in solution in the sap.

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TABLE VI.—MOLECULAR WEIGHTS OF PLANT SAP SOLUTES.

Date	Plant	Tissue	Greatest molecular weight	Smallest molecular weight
6–21–09	Elberta peach	Twig	154	154
6–24–09	Elberta peach	Twig	171	170
6–25–10	Elberta peach (cut back)	Twig	156	155
7–14–10	Elberta peach	Twig	159	158
7–27–10	Brigg's Red peach	Twig	180	179
7–27–10	Brigg's Red peach (cut back)	Twig	159	158
7–29–10	Elberta peach (cut back)	Twig	161	160
7–29–10	Elberta peach	Twig	186	186
8–30–09	Elberta peach	Twig	172	172
9– 9–10	Elberta peach (cut back)	Twig	168	168
9– 9–10	Elberta peach	Twig	184	182
10–14–09	Oldmixon Free peach	Twig	193	192
10–14–09	Elberta peach	Twig	175	172
10–28–09	Oldmixon Free peach (cut back)	Twig	187	185
10–29–10	Elberta peach (cut back)	Twig	179	173
10–29–10	Elberta peach	Twig	184	176
11– 4–09	Elberta peach from Georgia	Twig	172	171
11– 5–09	Salway peach from Dixon, Mo.	Twig	161	158
11– 6–10	Bokhara peach (cut back)	Twig	175	173
11– 6–10	Bokhara peach	Twig	178	176
11– 6–09	Elberta peach	Twig	187	187
11–20–09	Oldmixon Free peach	Twig	187	185
11–20–09	Elberta peach	Twig	182	182
11–20–08	Elberta peach	Twig	170	169
3– 5–10	Elberta peach	Twig	184	183
3– 6–09	Elberta peach	Twig	172	...
3–18–09	Elberta seedling peach	Twig	193	193
3–20–09	Elberta peach from Doniphan, Mo.	Twig	169	168
3–20–09	Sneed peach from Doniphan, Mo.	Twig	194	193
3–20–09	Elberta peach	Twig	177	174
3–20–09	Mountain Rose peach	Twig	163	159
3–30–09	Elberta peach	Twig	156	155
3–30–09	Elberta peach from Morrill, Texas	Twig	171	170
4– 4–10	Elberta seedling peach	Twig	197	196
4–11–10	Snow seedling peach	Twig	180	179
4–16–09	Elberta peach from Florida	Twig	129	129
4–18–10	Elberta peach from Georgia	Twig	191	...
4–19–09	Elberta peach from Georgia	Twig	192	191
4–23–09	Elberta seedling peach	Twig	170	170
5– 9–10	Family Favorite seedling peach	Twig	177	174
5– 9–10	Snow seedling peach	Twig	164	158
5– 9–10	Snow seedling peach	Twig	179	177
5– 9–10	Family Favorite seedling peach	Twig	180	177
5–13–11	Oldmixon Cling peach	Twig	151	150
5–18–11	Elberta peach	Twig	167	167
6– 7–09	Snow peach	Twig	159	156
6–10–09	Snow peach	Twig	167	167
11–21–08	Snow peach	Twig	180	175
11–26–10	Elberta peach	Twig	181	179
11–26–10	Oldmixon Cling peach	Twig	183	180
11–26–10	Elberta peach (cut back)	Twig	170	168
12– 3–08	Champion peach from Dixon, Mo.	Twig	194	193

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Date	Plant	Tissue	Greatest molecular weight	Smallest molecular weight
12– 3–08	Elberta peach from Geneva, N.Y.	Twig	185	182
12–11–08	Bruno & Krummel peach	Twig	187	187
12–12–08	Elberta peach from Brandsville, Mo.	Twig	195	188
12–12–08	Elberta peach from Brandsville, Mo.	Twig	195	185
12–28–09	Elberta peach from Doniphan, Mo.	Twig	179	174
12–28–09	Elberta peach from Brandsville, Mo.	Twig	181	181
1– 2–09	Elberta peach from Geneva, N. Y.	Twig	202	200
1– 2–09	Elberta peach	Twig	191	187
1– 2–11	Oldmixon Cling peach	Twig	163	161
1– 3–10	Elberta peach	Twig	174	171
1– 9–09	Elberta peach from Oklahoma	Twig	182	177
1–12–10	Elberta peach	Twig	178	171
2–13–09	Bruno & Krummel peach	Twig	159	159
2–16–11	Oldmixon Cling peach	Twig	166	165
2–20–09	Champion peach from Dixon, Mo.	Twig	170	167
2–27–08	Elberta peach	Twig	170	...
2–27–08	Elberta peach	Twig	172	...
2–27–09	Elberta peach	Twig	173	...
3–18–09	Elberta peach seedling	Fruit buds	148	147
11–20–09	Elberta peach	Fruit buds	187	186
11–21–09	Elberta peach	Fruit buds	172	...
6–25–09	Elberta peach	Seed kernel	113	113
4–18–09	Elberta peach	Leaf	151	151
6– 7–09	Snow peach	Leaf	145	144
6– 8–09	Family Favorite peach	Leaf	144	144
6–21–09	Elberta peach	Leaf	138	137
6–21–09	Elberta peach	Leaf	133	131
6–21–09	Elberta peach (cut back)	Leaf	128	128
6–24–10	Elberta peach	Leaf	151	146
7–15–10	Elberta peach (cut back)	Leaf	139	138
7–15–10	Elberta peach	Leaf	144	143
7–28–10	Brigg's Red peach (cut back)	Leaf	142	141
7–28–10	Brigg's Red peach	Leaf	156	153
7–30–10	Elberta peach (cut back)	Leaf	138	137
7–30–09	Elberta peach	Leaf	151	151
8– 9–10	Elberta peach (cut back)	Leaf	138	137
9– 9–10	Elberta peach (cut back)	Leaf	150	149
3– 5–10	Family Favorite seedling peach	Root	213	206
4– 4–10	Snow seedling peach	Root	222	217
5– 9–10	Family Favorite seedling peach	Root	255	254
5– 9–10	Snow seedling peach	Root	217	217
5– 9–10	Family Favorite seedling peach	Root	222	221
5– 9–10	Snow seedling peach	Root	222	221
6– 7–09	Snow peach	Root	204	202
6– 8–09	Family Favorite peach	Root	238	237
6–19–09	Elberta peach	Root	276	272
6–25–10	Elberta peach (cut back)	Root	362	361
6–25–10	Elberta peach	Root	386	385
6–28–09	Salway peach (cut back)	Root	314	310

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Date	Plant	Tissue	Greatest molecular weight	Smallest molecular weight
6–28–09	Salway peach	Root	318	316
7–14–09	Lemon Cling peach (cut back)	Root	434	420
7–14–09	Lemon Cling peach	Root	363	353
7–17–09	Elberta Seedling peach	Root	249	248
10–12–09	Oldmixon Free peach	Root	270	269
10–14–09	Oldmixon Free peach	Root	283	281
10–20–09	Snow seedling peach	Root	216	215
3– 5–10	Gano apple	Twig	193	191
4–18–10	Gano apple	Twig	163	162
4–25–10	Holt apple	Twig	174	173
6–10–09	Gano apple	Twig	180	180
6–22–10	Jonathan apple	Twig	180	179
6–24–10	Longevity apple	Twig	180	179
7–25–10	Jonathan apple	Twig	172	172
8–30–10	Jonathan apple	Twig	188	187
11–26–10	Jonathan apple	Twig	177	176
12–26–10	Jonathan apple	Twig	174	170
1– 1–11	Jonathan apple	Twig	170	164
4–25–10	Holt apple	Leaf	150	149
5–21–10	Jonathan apple	Leaf	159	156
6–10–09	Gano apple	Leaf	162	158
6–14–09	Gano apple	Leaf	174	172
6–21–09	Jonathan apple	Leaf	176	175
6–24–10	Longevity apple	Leaf	162	159
7–25–10	Jonathan apple	Leaf	179	179
6–14–09	Gano apple	Fruit	132	132
10–28–09	Gano apple	Fruit	143	139
3–10–10	Metett apple	Trunk	264	263
6–14–10	Gano apple	Trunk	245	243
6–24–10	Longevity apple	Trunk	247	246
3–10–10	Metett apple	Secondary branches	214	214
4–25–10	Holt apple	Secondary branches	207	207
6–14–09	Gano apple	Secondary branches	225	225
3–10–10	Metett apple	Primary branches	257	248
5–25–10	Holt apple	Primary branches	235	234
3– 5–10	Gano apple	Root	158	149
3–10–10	Metett apple	Root	187	186
3–22–10	Red Astrachan apple	Root	164	159
3–22–10	Huntsman apple	Root	171	170
3–22–10	Red Astrachan apple	Root	164	164
3–22–10	Huntsman apple	Root	171	170
3–22–10	Shiawassa apple	Root	167	166
3–22–10	Shiawassa apple	Root	160	159
3–22–10	Shiawassa apple	Root	181	180
3–22–10	Huntsman apple	Root	173	172
6– 9–09	Gano apple	Root	165	164
7–10–09	Gano apple	Root	163	161
8–10–09	Gano apple (all leaves removed)	Root	189	188

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Date	Plant	Tissue	Greatest molecular weight	Smallest molecular weight
9–10–09	Ben Davis apple (girdled)	Root	160	158
10–10–09	Ben Davis apple	Root	170	168
10–28–09	Gano apple	Root	167	164
11– 4–09	Huntsman apple	Root	167	167
11– 6–09	Huntsman apple	Root	176	175
11–17–09	Jonathan apple	Root	157	156
1–18–10	Jonathan apple	Root	158	157
6–26–09	Prunus Domestica plum	Leaves	153	149
6–26–09	Prunus Domestica plum	Fruit	126	125
5–23–09	Morello cherry	Fruit	296	292
5–23–10	Silver maple, Acer saccharinum Linn.	Leaf	178	177
5–23–10	Silver maple, Acer saccharinum Linn.	Twig	190	187
6–23–10	Silver maple, Acer saccharinum Linn.	Twig	212	211
5–23–10	Silver maple, Acer saccharinum Linn.	Secondary branch	255	253
5–23–10	Silver maple, Acer saccharinum Linn.	High trunk	389	385
5–23–10	Silver maple, Acer saccharinum Linn.	Main branch	327	327
5–23–10	Silver maple, Acer saccharinum Linn.	Root	305	302
6–23–10	Silver maple, Acer saccharinum Linn.	Root	314	314
3– 8–10	Silver maple, Acer saccharinum Linn.	Trunk (tapped)	265	262
5–27–09	Elm, Ulmus Americana, Linn.	Leaf	152	150
5–27–09	Elm, Ulmus Americana, Linn.	Root	192	192
6–23–09	Asparagus	Root	234	231
8–13–09	Asparagus	Root	288	286
9–18–09	Asparagus	Root	256	252
11–11–11	Asparagus	Root	289	288

It will be noted that the solute from the cortex sap of Elberta twigs from Florida was much lower than that of the cortex of any other twigs listed. These twigs were in a very unnatural, exhausted condition due to their growth in a section further south than that in which peaches of the Elberta type reach their best development. It seems possible that the low molecular weight might be explained by the fact that the winter was warm and respiration rapid throughout the dormant season. Hence the sugar and associated compounds would be largely exhausted, leaving compounds, especially electrolytes, of low molecular weights. However, twigs from Morrill, Texas, did not show this low molecular weight, yet the twigs were, to some extent, in the same condition as those from Florida.

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In the case of apples the leaf and fruit saps show low molecular weight. The roots show molecular weight about equal to that of the twigs, certainly no higher. On the other hand, the few determinations made indicate that sap of the cortex of the large branches and the trunk of the tree contains a solute with a higher molecular weight than that of any of the other tissues from the apple tree.

The one determination made from the fruit sap of the cherry indicates a very high molecular weight, but a single determination has little value.

In the case of the silver maple, *Acer saccharinum* Linn., the sap from the leaf shows the lowest molecular weight of the solute, that from the twigs next, that from the roots next, that from the main and secondary branches next, and from the trunk the highest, considering cortex sap only. The sap from the sap wood of the silver maple tree, obtained by tapping, has apparently a much lower molecular weight than that from the cortex.

SAP DENSITY OF VARIOUS TISSUES OF TREES AS SHOWN BY FREEZING POINT DETERMINATIONS.

Freezing point determinations were made from cortex sap of apple trees. Samples were selected from various regions of the tree both below and above ground.[1] The following table gives the results of these determinations. The figures are given in terms of depression, meaning the number of degrees Centigrade below the freezing point of water at which these saps began to freeze.

The above figures show, with some exceptions, that during the summer season beginning here about May 1 the molar concentration of the cortex sap was lowest near the ground and highest in the upper parts of the tree. In all cases it was considerably the highest in the leaves and generally lowest in the roots. With apples this does not seem to be true during a period beginning in December and continuing until about May 1. In March and April, especially while the sap density of the roots is generally lowest, usually the trunk cortex has a higher sap density than the cortex of the twig. It will be noted in the cases of the elm and sycamore, that in June, there was a steady increase

1. W. T. Sutherst, "The Freezing Point of Vegetable Saps and Juices," Chem. News, Vol. LXXXIV (1901), p. 234.
H. H. Dixon and W. R. G. Atkins, "Osmotic Pressures in Plants; and a Thermo-electric Method of Determining Freezing Points." Sci. Proc. Roy. Dublin Soc., n. ser., 12 (1910), No. 25, pp. 275-311.
H. H. Dixon and W. R. G. Atkins, "Changes in the Osmotic Pressure of the Sap of Leaves of Various Plants," Sci. Proc. Roy. Dublin Soc. n. ser. 13 (1912), Nos. 16:219-222; 18:229-238; 19:239-246.

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TABLE VII.—SAP DENSITIES OF VARIOUS TISSUES OF TREES AS SHOWN BY FREEZING POINT DEPRESSIONS.

Date	Material	Roots	Trunk	Main branches	Secondary branches	Twigs	Leaves	Fruit
3- 9-10	Metett apple	1.391	1.848	1.923	1.738	1.768	.....	.....
3-11-10	Pasman apple	1.477	2.268	.....	1.818	1.698	.....	.....
4- 8-11	Regan apple	1.175	1.590	1.375	1.275	1.270	.....	.....
4-22-10	Holt apple	.995	1.005	.980	.740	.925	1.325	.....
5- 9-11	Regan apple	.965	1.175	1.065	1.290	1.320	1.690	.....
5-25-10	Kauser apple	.835	.905	.960	.790	.920	1.555	.....
6-10-11	Bloomfield apple	1.215	1.370	1.470	1.580	1.930	2.230	.....
6-15-09	Gano apple	.927	1.092	1.112	1.132	1.420	2.134	1.007
6-23-10	Longevity apple	.933	1.133	1.153	1.198	1.343	2.068	.....
7-12-10	Gano apple	.965	.....	.....	.....	1.214	1.917	1.033
7-13-09	Bloomfield apple	1.265	.....	1.695	1.905	2.595	2.540	.....
8- 3-09	Gano apple	1.015	.....	.....	.....	1.590	2.632	1.247
8- 5-11	Gano apple	1.245	.....	1.230	.....	1.310	2.065	1.495
10-23-09	Lawver apple	1.321	1.399	1.557	1.582	1.803	.....	.....
11-13-09	Wallace apple	1.211	1.450	1.349	.....	1.540	.....	.....
6-15-11	Snow peach	1.025	.....	1.470	1.485	1.205	1.990	.....
6-19-11	Foster peach	1.155	.....	1.365	1.750	1.955	2.645	.....
6-19-11	Foster peach (vigorous)	1.065	.....	1.245	1.315	1.745	2.200	.....
7-10-11	Seedling peach	1.530	1.660	1.740	1.780	2.270	.....	.....
8- 3-11	Elberta peach	1.470	.....	.....	1.760	1.860	3.090	.....
8- 8-11	Snow's Orange peach	1.395	.....	2.550	2.140	2.580	3.150	.....
8- 8-11	Snow's Orange peach	1.425	.....	2.235	1.910	2.140	3.040	.....
8-10-11	Foster peach	1.285	.....	1.825	2.545	2.395	3.015	.....
8-10-11	Foster peach (vigorous)	1.245	.....	1.825	2.240	2.185	2.840	.....
8-14-11	Three seedling peach	1.365	1.546	1.631	1.531	2.086	.....	.....
9- 2-11	Three seedling peach	1.190	1.460	.....	1.460	1.695	.....	.....
7- 9-12	Seedling peach	1.130	1.252	1.275	1.495	1.728	2.390	.....
5-22-10	Silver maple	.570	.620	.600	.550	.705	.965	.....
6-22-10	Silver maple	.585	.645	.920	.965	1.155	1.360	.....
6-29-10	Sycamore	.525	.575	.675	.865	.995	1.170	.....
6-29-10	Elm	.495	.475	.715	.720	.995	1.360	.....
7-25-12	Lily of Kent apple	1.120	1.182	1.254	1.567	1.538	1.917	1.317
9- 2-12	Early Harvest apple	.....	1.313	1.542	1.672	1.740	.....	.....
9-17-12	Lily of Kent apple	1.105	1.415	1.292	1.361	1.488	1.955	1.530
12- 2-12	Lily of Kent apple	1.420	1.930	1.665	1.500	2.000	.....	.....

in the cortex sap concentration from the ground upward in the tree. though this was not so uniform in the case of the silver maple. On March 5, 1910, sap was secured from the sap wood of the silver maple by boring a hole into the sap wood and collecting the exuding sap in a test tube. The depression for such sap was:

From large surface roots.....0.143
From low on the trunk.....0.127

The depression for sap taken in the same way on March 13th was:

From large surface roots.....0.265
From low on the trunk.....0.290
From eight feet up the trunk.....0.317

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Sap determinations were taken from sections of the cortex of a large, healthy apple tree, beginning at the extremity of a large root and continuing up to the top of the tree. The trunk cortex was taken from over the roll of sap wood that connected this root with a branch above, and the branch cortex was taken from the branch that plainly connected through the sap wood with the large root mentioned. The twigs and leaves were also taken from this branch. The following table gives the results:

TABLE VIII.—FREEZING POINT DEPRESSIONS OF SAP FROM CORTEX OF APPLE TREE TISSUES, JULY 24, 1913.

Tissue	Distance from crown	Average diameter	Depression
Root	25 to 28 ft.	3-16 in.	1.272
Root	21 to 24 ft.	1-4 in.	1.105
Root	17 to 20 ft.	3-8 in.	1.255
Root	15 to 16 ft.	5-8 in.	1.232
Root	13 to 14 ft.	7-8 in.	1.207
Root	11 to 12 ft.	1 in.	1.200
Root	9 to 10 ft.	1 in.	1.128
Root	7 to 8 ft.	1 1-8 in.	1.218
Root	5 to 6 ft.	1 3-8 in.	1.197
Root	2 to 4 ft.	1 1-2 in.	1.185
Trunk	1 ft.	12 in.	1.355
Trunk	2 ft.	10 in.	1.418
Branches	4 ft.	6 in.	1.272
Branches	6 ft.	5 in.	1.365
Branches	8 ft.	4 1-2 in.	1.360
Branches	10 ft.	3 1-2 in.	1.350
Branches	12 ft.	3 in.	1.402
Branches	14 to 15 ft.	3 in.	1.475
Branches	16 to 17 ft.	1 1-2 in.	1.542
Branches	18 to 19 ft.	7-8 in.	1.650
Branches	20 to 23 ft.	3-8 in.	1.850
Branches	current season's growth	3-16 in.	1.825
Leaves			2.197

There was no constant difference in the molar concentration of the root cortex sap at that date. The sap from the trunk cortex two feet above the ground was more dense than that from sections twelve or fifteen feet above ground. There was a steady rise in concentration from four feet above ground to the leaves.

Determinations were made from sections of a long root from this same tree on January 15 and of another on February 24, 1913. Following are the depressions:

It will be seen that in January and February the depressions were greater near the trunk. The molar concentration of the tissue near the trunk was much greater at this time than in July.

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TABLE IX.—FREEZING POINT DEPRESSION OF SAP FROM CORTEX OF APPLE ROOT.

Date	Distance from crown	Depression
1–16–13	1 ft.	1.595
	2 ft.	1.600
	3 ft.	1.620
	4 ft.	1.655
	5 ft.	1.305
	6 ft.	1.285
	7 ft.	1.272
	8 ft.	1.265
	9 ft.	1.315
	10 ft.	1.240
	11 ft.	1.145
2–24–13	1 to 2 ft.	1.383
	3 to 4 ft.	1.388
	5 to 6 ft.	1.325
	7 to 8 ft.	1.325
	9 to 10 ft.	1.325
	11 to 12 ft.	1.250
	13 to 14 ft.	1.290
	15 to 16 ft.	1.290
	17 to 18 ft.	1.225
	19 to 20 ft.	1.250

SAP CONCENTRATION AT DIFFERENT PERIODS OF THE YEAR.

The data in Table VII where samples were taken from the same species during enough different seasons of the year to be conclusive, suggest that the molar concentration of the cortex sap is smallest in late May and June, the most rapidly growing period.

Many more freezing point determinations were made of sap from the cortex of twigs than from any other tissue. The following table, showing these determinations, makes it evident that there was a considerably lower molar concentration in the cortex sap during the early summer period when the trees are growing rapidly, than during the later summer and winter periods.

It is interesting to note that the reduced concentration comes earlier in the season, and the increase in concentration begins earlier in the summer in the case of the apple than with the peach. This may be explained by the fact that the peach continues rapid growth later in the season than the apple. Exceptions might possibly come in the case of very young apple trees, but all of the trees listed here were of bearing age.

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TABLE X.—VARIATIONS IN FREEZING POINT DEPRESSIONS OF TWIG SAPS.

Date	Gano apple	Jonathan apple	Elberta peach
3- 5-10	1.616	.....	.....
3- 5-10	.....	.....	1.667
3-30-09	.....	.....	1.440
3-30-09	.....	.....	1.848
4-11-10	.978	.....	.....
4-15-10	.920	.....	.....
4-17-09	.....	.....	1.914
4-17-09	.....	.....	1.937
4-19-09	.....	.....	1.804
4-20-10	.....	1.055	.....
4-21-10	.....	.....	1.275
4-26-09	.....	.....	1.915
5- 9-10	1.055	.....	.....
5-20-10	1.115	.....	.....
5-21-10	.....	.....	1.055
5-21-10	.....	.915	.....
6-10-09	1.217	.....	.....
6-15-10	1.360	.....	.....
6-21-10	.....	1.415	.....
6-21-09	.....	.....	1.285
6-21-09	.....	.....	1.465
6-22-10	.....	.....	1.310
6-27-10	.....	.....	1.095
6-28-09	.....	.....	1.162
7- 6-09	.....	.....	1.150
7-10-09	1.213	.....	.....
7-14-10	.....	.....	1.280
7-14-10	.....	.....	1.355
7-18-10	1.725	.....	.....
7-25-10	.....	1.500	.....
8- 3-09	1.340	.....	.....
8- 5-11	1.295	.....	.....
8-10-10	.....	.....	1.475
8-10-10	.....	.....	1.695
8-17-09	.....	.....	1.628
8-17-09	.....	1.672	.....
8-19-10	1.800	.....	.....
8-25-10	.....	1.505	.....
8-30-09	.....	1.691	.....
8-30-09	.....	.....	1.813
9- 1-08	.....	.....	2.037
9- 2-08	.....	.....	2.161
9- 2-11	.....	.....	1.970
9- 4-10	.....	.....	1.970
9- 7-10	.....	.....	1.515
9- 7-10	.....	.....	1.645
9-15-09	1.690	.....	.....
9-15-11	.....	1.605	.....
9-30-11	.....	.....	1.865
10- 1-10	.....	.....	1.680
10- 1-10	.....	.....	1.710
10- 8-10	.....	1.870	.....
10-13-09	.....	.....	1.948
10-14-09	.....	.....	1.845
10-16-09	1.811	.....	.....

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Date	Gano apple	Jonathan apple	Elberta peach
10–21–11	.....	1.995	.....
10–22–10	.....	.....	1.605
10–22–10	.....	.....	1.620
10–27–11	.....	.....	2.055
10–28–09	1.728	.....	.....
10–28–09	.....	.....	1.819
10–28–09	.....	.....	2.010
10–29–10	.....	.....	1.628
10–29–10	.....	.....	1.528
11– 5–08	.....	.....	2.220
11– 6–08	.....	.....	2.320
11–11–11	.....	.....	1.925
11–14–08	.....	.....	2.088
11–17–09	.....	1.912	.....
11–19–09	.....	.....	1.870
11–19–09	.....	.....	1.863
11–23–10	1.655	.....	.....
11–25–10	.....	.....	1.665
11–25–10	.....	.....	1.705
11–25–10	.....	1.935	.....
12– 8–09	.....	.....	1.694
12–16–09	.....	2.016	.....
1– 1–09	.....	.....	2.525
1– 1–09	.....	.....	2.525
1– 1–11	.....	2.095	.....
1–12–10	.....	.....	1.535
1–19–10	.....	2.013	.....
1–21–10	.....	.....	1.645
1–27–10	1.630	.....	.....
2–22–10	.....	2.170	.....
2–26–10	.....	1.734	.....

SAP CONCENTRATION OF GREEN AND RIPE APPLE AND PEACH FRUITS.

The following table gives the molar concentration of the fruit saps of apple and peach in terms of the depression of the freezing point. The first calculations were made when the fruit was very small and subsequent ones at various intervals until the fruit was ripe.

In case of both the apple and peach the concentration was greater when the fruit was first set than it was later when the fruit was larger and probably making more rapid growth. When the fruit was ripe, however, it had a greater concentration than at any time previous. This would be expected. In the case of peaches, the sap of ripe fruit of late varieties such as Kalamazoo, Boley Free, Dewey and Hubert Cling, had a considerably higher molar concentration than that of ripe fruits of earlier varieties like Lewis, Yellow St. John, Alexander and Sneed.

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TABLE XI.—FREEZING POINT DEPRESSION OF FRUIT SAPS.

Date	Fruit	Variety	Sap of unripe fruit	Sap of ripe fruit
5– 4–09	Apple	Gano	1.624	
5– 4–09	Apple	Gano	1.547	
5–11–11	Apple	Ben Davis	1.250	
6–13–11	Apple	Ben Davis	1.230	
7–12–09	Apple	Gano	1.046	
7–19–11	Apple	Gano	1.465	
7–22–09	Apple	Ben Davis	1.155	
7–22–09	Apple	Grimes Golden	1.273	
7–24–11	Apple	Gano	1.430	
8– 3–09	Apple	Gano	1.292	
8–15–11	Apple	Gano	1.495	
9–29–09	Apple	Gano		1.823
12– 2–11	Apple	Gano		1.950
4–18–10	Peach	Seedling Elberta	1.130	
4–18–10	Peach	Elberta	1.395	
4–22–10	Peach	Seedling Elberta	1.105	
5–11–10	Peach	Elberta	1.205	
6–12–10	Peach	Elberta	1.073	
6–12–10	Peach	Elberta	1.077	
6–12–10	Peach	Elberta	1.078	
6–12–10	Peach	Elberta	1.165	
6–12–10	Peach	Sneed	1.042	
6–13–10	Peach	Alexander	1.185	
6–17–08	Peach	Elberta	1.117	
6–17–09	Peach	Elberta	1.005	
6–21–08	Peach	Sneed		1.165
6–18–08	Peach	Sneed	.961	
6–21–08	Peach	Alexander	1.012	
6–24–09	Peach	Elberta	1.091	
6–25–08	Peach	Sneed		1.105
6–25–08	Peach	Lewis	1.193	
6–25–08	Peach	Alexander	1.080	
6–30–08	Peach	Triumph	1.213	
7– 1–08	Peach	Alexander	1.068	1.165
7– 8–08	Peach	Triumph	1.304	1.474
7–17–11	Peach	Alexander		1.210
7–18–08	Peach	Lewis	1.307	1.464
7–18–08	Peach	Yellow St. John	1.287	1.320
7–18–08	Peach	Rareripe	1.328	1.340
7–29–08	Peach	Lewis		1.357
8–14–08	Peach	Elberta	1.289	
8–22–08	Peach	Kalamazoo		1.824
8–27–08	Peach	Boley Free		1.734
8–31–08	Peach	Dewey Cling		1.959
8–31–08	Peach	Hubert Cling		1.677

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EFFECT OF VIGOROUS GROWTH ON MOLAR CONCENTRATION OF SAP.

The figures indicate that at the time the molar concentration is least, trees are making most rapid growth. Further data have been obtained [1] to indicate that vigorous growth generally results in a lower sap density. In the spring and winter preceding the seasons of 1909, 1910 and 1911, some peach trees in the Missouri Experiment Station orchard were cut back rather severely. One-half to two-thirds of the tops of these trees were cut off. The tops of other trees of the same variety were pruned but slightly. In the tables the trees will be designated as "Cut back" and "Not cut back." Of course during the season following, the trees that were cut back made a much more vigorous growth generally and continued to grow later in the summer than did the trees that were not cut back. The following table gives the freezing point depression of sap from various tissues of trees that were cut back and those that were not cut back, the sap being taken at intervals during the summer following the pruning:

It will be seen that except in the early growing season when the cutting back could not yet have influenced the sap, the molar concentration was generally lower in the tissues of cut back trees than in those which were not cut back. There are some exceptions in the latter part of the summer. This, however, is probably explained by the fact that some of the trees were rather seriously injured by having so much of their root system, and also the top, removed for the earlier determinations. In October and November the concentration is again about the same for each group of trees. This is probably due to a long season giving the cut back trees time for storage even after late growth.

Molar concentration determinations from roots of asparagus made throughout the season also tend to show that during the season of rapid growth the roots, at least, show reduced concentration. The young shoots of asparagus in garden practice are cut for use from the time they start in the spring, until about June 15th. Following the time at which cutting ceases, there is a period in which growth of new shoots is very rapid. The cutting of asparagus should tend to reduce the drain on the stored nutrients of the roots, since cutting the young

1. E. B. Copeland, The Relation of Nutrient Salts to Turgor. Bot. Gaz. 24, 399-416. 1897.
H. H. Dixon and W. R. G. Atkins, Changes in the Osmotic Pressure of the Sap of the Leaves of Various Plants. Sci. Proc. Roy. Dublin Soc. n. ser., 1912, 13, 219-222; 18, 229-238; 19, 239-246.

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TABLE XII.—DEPRESSION OF SAP FROM TISSUES OF CUT BACK TREES AND TREES NOT CUT BACK.

Date	Variety	Tissue	Depression trees not cut back	Depression trees cut back
4–14–11	Early Michigan peach	Root	1.293	1.208
4–17–11	Early Tillotson peach	Root	1.265	1.300
4–17–11	Bell's October peach	Root	1.285	1.295
4–15–10	Early Tillotson peach	Root	.967	1.047
4–15–10	Early Michigan peach	Root	.997	1.137
4–15–10	Bell's October peach	Root	1.337	1.322
5–13–10	Early Tillotson peach	Root	.975	1.055
5–13–10	Bell's October peach	Root	.945	1.105
5–14–10	Early Michigan peach	Root	.985	.965
6–15–10	Snow's Orange peach	Root	1.140	1.025
6–17–10	Early Tillotson peach	Root	.965	.830
6–17–10	Bell's October peach	Root	.865	.785
6–17–10	Early Michigan peach	Root	.875	.855
6–19–11	Foster peach	Root	1.155	1.065
6–24–10	Elberta peach	Root	.910	.830
7– 5–10	Briggs Red peach	Root	.910	.790
7–14–10	Elberta peach	Root	.960	.905
7–16–10	Early Michigan peach	Root	1.030	.780
7–16–10	Early Tillotson peach	Root	1.085	1.000
7–16–10	Bell's October peach	Root	1.030	.840
8– 8–11	Snow's Orange peach	Root	1.395	1.425
8–10–11	Foster peach	Root	1.285	1.245
8–30–10	Early Tillotson peach	Root	1.280	1.360
8–30–10	Bell's October peach	Root	1.265	1.240
8–31–10	Elberta peach	Root	1.200	1.075
9– 2–10	Early Michigan peach	Root	1.300	1.140
9– 2–10	Bokhara peach	Root	1.180	1.076
9– 2–10	Globe peach	Root	1.145	.950
9– 2–10	Heath Cling peach	Root	1.140	.955
10– 5–09	Oldmixon Free peach	Root	1.257	1.243
11–20–09	Oldmixon Free peach	Root	1.423	1.517
6–15–11	Snow's Orange peach	Twig	1.490	1.205
6–19–11	Foster peach	Twig	1.955	1.745
7– 5–10	Brigg's Red peach	Twig	1.600	1.300
7–14–10	Elberta peach	Twig	1.345	1.280
7–27–10	Brigg's Red peach	Twig	1.658	1.348
7–28–10	Bokhara peach	Twig	1.555	1.335
7–29–10	Elberta peach	Twig	1.385	1.213
8– 8–11	Snow's Orange peach	Twig	2.580	2.140
8–10–11	Foster peach	Twig	2.395	2.185
8–10–10	Elberta peach	Twig	1.725	1.505
9– 7–10	Elberta peach	Twig	1.655	1.525
10– 1–10	Elberta peach	Twig	1.705	1.675
10–11–10	Early Michigan peach	Twig	1.725	1.700
10–12–09	Oldmixon Free peach	Twig	1.728	1.916
10–22–10	Elberta peach	Twig	1.620	1.605
10–28–09	Oldmixon Free peach	Twig	2.019	1.819
10–29–10	Elberta peach	Twig	1.628	1.528
11– 5–10	Bokhara peach	Twig	2.290	1.817
11–19–10	Bell's October peach	Twig	1.963	2.063
11–19–09	Oldmixon Free peach	Twig	1.870	1.818

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Date	Variety	Tissue	Depression trees not cut back	Depression trees cut back
11–25–10	Elberta peach	Twig	1.705	1.665
6–15–11	Snow's Orange peach	Leaf	1.990	2.350
6–19–11	Foster peach	Leaf	2.200	2.645
7–14–10	Elberta peach	Leaf	2.545	2.275
7–27–10	Brigg's Red peach	Leaf	2.428	1.928
7–28–10	Bokhara peach	Leaf	2.545	2.115
7–28–10	Elberta peach	Leaf	2.170	2.110
8– 8–10	Snow's Orange peach	Leaf	3.040	3.135
8–10–10	Elberta peach	Leaf	2.875	2.620
8–22–09	Foster peach	Leaf	3.015	2.840
9–22–10	Elberta peach	Leaf	3.510	2.403
10– 7–10	Elberta peach	Leaf	2.815	2.510
10–22–10	Elberta peach	Leaf	3.455	3.295
10–11–10	Early Michigan peach	Leaf	3.275	3.050
10– 5–10	Bokhara peach	Leaf	2.785	2.895
6–15–11	Snow's Orange peach	Main branch	2.055	1.740
6–19–11	Foster peach	Main branch	1.365	1.245
8– 8–11	Snow's Orange peach	Main branch	2.550	2.235
8–10–11	Foster peach	Main branch	1.825	1.825
6–15–11	Snow's Orange peach	Secondary branches	1.820	1.485
6–19–11	Foster peach	Secondary branches	1.750	1.315
8– 8–10	Snow's Orange peach	Secondary branches	2.140	1.910
8–10–10	Foster peach	Secondary branches	2.545	2.240

shoot checks growth until a new shoot starts. The following table gives the average molar concentration of the sap of asparagus roots in terms of the freezing point depression for each month in the year. Generally a considerable number of determinations were made for each month. The average moisture content for each month is also given, to indicate the effect the difference in moisture content might have on the greater concentration during winter and early spring months.

The amount of water in the tissues apparently influenced the molar concentration to some extent but not enough to affect the trend of the results.

It is interesting to note that apparently the lowest molar concentration is during the period after cutting has ceased when there is very rapid growth of the new shoots without the checking of

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TABLE XIII.—DEPRESSION OF ASPARAGUS ROOT SAP FOR EACH MONTH.

Month	Depression	Moisture content
March	1.525	71.3%
April	1.362	.....
May	.988	83.3%
June	.922	85.4%
July	.784	87.5%
August	.850	85.3%
September	1.003	84.3%
October	1.163	82.7%
November	1.190	83.4%
December	1.216	82.2%
February	1.324	80.3%

growth by cutting such as we have just mentioned. Apparently this growth is made largely from the plant food stored in the roots. If this is true and more growth is made when the asparagus is left uncut, the roots of cut asparagus should show the highest concentration. The following table shows that this is apparently true.

TABLE XIV.—MOLAR CONCENTRATION OF ROOT SAP FROM CUT AND UNCUT ASPARAGUS.*

Date	Depression uncut	Depression cut
6–28–10	.930	1.000
6–28–10	.815	.895
6–28–10	.890	.950
7– 5–10	.885	.810
7– 5–10	.625	.930
7– 5–10	.675	.875
7–12–10	.778	.888
7–12–10	.703	.803
7–12–10	.818	.958

While the molar concentration of sap from the roots of uncut asparagus is lower immediately following the cutting season than that from cut asparagus, the following table indicates that the reverse is true for the stems.

*In the case of uncut asparagus, cutting ceased June 28 while it continued in the case of cut asparagus.

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TABLE XV.—MOLAR CONCENTRATION OF STEM SAP OF CUT AND UNCUT ASPARAGUS.

Date	Depression uncut	Depression cut
6-28-10	.790	.700
6-28-10	.760	.775
6-28-10	.770	.765
7- 5-10	.870	.700
7- 5-10	1.015	.745
7- 5-10	.980	.735
7-12-10	1.153	.713
7-12-10	1.008	.753
7-12-10	1.088	.718

MOLAR CONCENTRATION AS A MEASURE OF THE NUTRITION OF THE ROOTS.

The molar concentration seems to be a fair measure of the condition of nutrition of the roots. When a tree is girdled, that is, a ring of bark removed from the tree so that the products of photosynthesis can not pass down into the roots, it is well known that the roots are left in a starved condition. The reserved plant food is then gradually drawn upon, while on the other hand, the size of the fruit, etc., at the top may be increased due to the retention above the girdle of the plant

TABLE XVI.—FREEZING POINT DEPRESSION OF SAP FROM GIRDLED AND UNGIRELED TREES.

Variety	Date tree was girdled	Date sap was frozen	Section of tree	Depressions girdled	Depressions not girdled
Garry Holden peach	Early	5–28–10	Root	.760	.790
Garry Holden peach	Early	6–30–10	Root	.895	1.040
Garry Holden peach	Early	7–23–10	Root	.835	1.040
Elberta peach	6– 5–11	8– 3–11	Root	.650	1.470
Elberta peach	6– 5–11	8– 3–11	Trunk and main branches	1.900	1.430
Elberta peach	6– 5–11	8– 3–11	Intermediate branches	2.140	1.760
Elberta peach	6– 5–11	8– 3–11	Twigs	2.020	1.860
Elberta peach	6– 5–11	8– 3–11	Leaves	3.410	3.090
Ben Davis apple	9–10–09	9–10–09	Root	1.385	1.450
Ben Davis apple	9–10–09	9–25–09	Root	1.206	1.339
Ben Davis apple	9–10–09	10–23–09	Root	1.295	1.381
Ben Davis apple	9–10–09	9–10–09	Twigs	1.625	1.661
Ben Davis apple	9–10–09	9–25–09	Twigs	1.848	1.606
Ben Davis apple	9–10–09	10–25–09	Twigs	1.660	1.402

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food that would normally go into the roots. The following table gives results of the determinations of the molar concentration of sap from various tissues of trees that had been girdled, and trees of the same variety that had not been girdled.

It will be seen that soon after the girdling, the starved condition of the roots can be detected by freezing point determinations of the cortex sap. Fruit trees from which the leaves had been removed contained a reduced supply of material in solution as the freezing point determinations of their sap will show.

TABLE XVII.—FREEZING POINT DEPRESSIONS OF SAPS FROM TREES NORMALLY GROWN COMPARED WITH THOSE FROM TREES FROM WHICH LEAVES HAD BEEN REMOVED.

Date on which leaves were removed	Date of freezing point determination	Species	Section of plant	Depression leaves not removed	Depression leaves removed
6– 1–10	6–16–10	Apple	Root	1.065	.745
6–13–09	6–17–09	Apple	Root	1.059	.991
6– 1–10	6–20–10	Apple	Root	.980	.770
6– 1–10	7–18–10	Apple	Root	1.110	.730
6– 1–10	8– 9–09	Apple	Root	1.477	1.047
6– 1–10	8–19–10	Apple	Root	1.055	.895
7–20–09	9– 6–09	Apple	Root	1.540	1.143
6– 1–10	11–23–10	Apple	Root	1.415	1.155
6– 1–10	6–16–10	Apple	Twig	1.500	.645
6– 1–10	6–20–10	Apple	Twig	1.390	.620
6– 1–10	7–18–10	Apple	Twig	1.725	.885
6– 1–10	8–19–10	Apple	Twig	1.800	1.345
6– 1–10	11–23–10	Apple	Twig	1.605	1.655
6– 7–11	7–10–11	Peach	Root	1.530	.820
6– 7–11	8–14–11	Peach	Root	1.366	1.151
6– 7–11	9– 2–11	Peach	Root	1.190	1.130
6– 7–11	7–10–11	Peach	Twig	2.270	1.285
6– 7–11	8–14–11	Peach	Twig	2.086	1.871
6– 7–11	9– 2–11	Peach	Twig	1.695	1.450
6– 7–11	7–10–11	Peach	Trunk	1.660	1.095
6– 7–11	8–14–11	Peach	Trunk	1.546	1.546
6– 7–11	9– 2–11	Peach	Trunk	1.460	1.320
6– 7–11	7–10–11	Peach	Main branch	1.740	.970
6– 7–11	8–14–11	Peach	Main branch	1.631	1.385
6– 7–11	7–10–11	Peach	Secondary branch	1.780	1.025
6– 7–11	8–14–11	Peach	Secondary branch	1.531	1.381
6– 7–11	9– 2–11	Peach	Intermediate branch	1.460	1.250

It will be seen that in all tissues the effect of the removal of the leaves can be detected in a short time by the reduced molar concentration of the sap. The greatest difference was detected shortly

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after the leaves were removed and before growth of new leaves was sufficient to affect the results.

These data and also those above on the density of sap of roots at the time of the most rapid growth of the top seem to indicate that the molar concentration determinations are fair indications of the nutrition of roots, especially when considered in connection with the apparent fact that there is reduced root growth at times of the greatest top growth.[1] Hammerle[2] found that the roots of *Acer pseudoplatanus* made their principal growth in spring and autumn with very slight growth in summer. This station has found sap density to be lowest in early summer.

Of interest in this line is the molar concentration of the cortex sap from Elberta peach twigs from Morrill, Texas. On March 30, 1909, twigs were taken by the writer at that point and carefully wrapped and sent back to Columbia, Missouri, for freezing point determination of the sap. The preceding winter had been so dry and warm that the trees in spring seemed almost too weak to send out the first foliage and bloom. Many of the blossoms dried up and fell prematurely from the tree. Although leaf growth was showing on Elberta at Columbia, yet at Morrill, 500 to 600 miles further south, leaf growth had not yet started. The freezing point depression of Elberta twig cortex sap from Morrill, was 1.002° C. while that from Columbia, was 1.400° C. to 1.848° C. Here also the molar concentration of the sap seemed to be a measure of the condition of the nutrition of the tree.

If molar concentration is a fair estimate of the nutrient condition of the plant tissue, then some interesting observations may be made from Table XI as to the effect of very vigorous pruning on the rate of growth of the top. It is very often stated that trees that have been pruned severely make vigorous growth because the plant food stored in the roots, trunks, and limbs of the tree is divided up among a smaller number of growing points and therefore more nutrients are available for each of the shoots left. When we consider, however, that nearly throughout the season the molar concentration of these vigorously growing, severely pruned trees is smaller than that of unpruned trees, it would seem rather doubtful if the increased food supply explains the more rapid growth of the twigs of the severely pruned trees. It would seem more likely that the late

1. Jost Phys. of Plts. Gibson Trans. p. 346.
2. Hammerle, Beitr. Wiss. Bot., 4, pt. 2, p. 15; abs. in Bot. Centlb., 88 (1901), No. 4, p. 107.

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vigorous growth of the twigs left on the pruned trees may be due to the more abundant water supply. When a tree is pruned severely there is a large root system to supply water for a smaller top. Not only does the normal root system have a much smaller top to supply, but the top is brought nearer the root system so that a great amount of resistance to the movement of water is eliminated.[1] That the evaporation from the top is probably very greatly reduced by the severe pruning may be seen from the following table which gives the total leaf surface, at the end of the season, of peach trees that had been pruned severely as compared with that of check trees which had not been pruned severely. The leaves were measured by making their outlines on cross section paper and counting the squares.

TABLE XVIII.—COMPARATIVE LEAF SURFACE OF PRUNED AND UNPRUNED PEACH TREES.

Variety	Number of leaves	Number of leaves measured	Average size of leaves	Total surface of tree
Oldmixon, Pruned.....	18,686	100	5.41 sq. in.	702.71 sq. ft.
Oldmixon, Unpruned.....	24,985	100	4.89 sq. in.	848.44 sq. ft.
Oldmixon, Unpruned.....	55,042	100	4.18 sq. in.	1,597.74 sq. ft.
Oldmixon, Pruned.....	18,245	100	4.71 sq. in.	599.76 sq. ft.
Oldmixon, Unpruned.....	32,286	100	5.20 sq. in.	1,165.80 sq. ft.
Elberta, Pruned.....	17,806	100	4.82 sq. in.	596.00 sq. ft.
Elberta, Pruned.....	14,077	100	5.25 sq. in.	513.22 sq. ft.
Elberta, Unpruned.....	30,096	100	4.75 sq. in.	992.75 sq. ft.

The evidence indicates that a pruned tree will not have as large a root system in the latter part of the season as it would have if the tree were left unpruned. Pickering[2] of the Woburn Experiment Station has found that in the English climate severe pruning is unquestionably a dwarfing process. This has been found true of peaches in Missouri. It seems probable that the explanation of this is a reduced root growth on account of the reduction of the leaf surface.

The fact that the roots have a lower concentration is suggestive of this condition. Although the rapidly growing tops have apparently a smaller concentration it should be remembered that at the time of the most rapid top growth there is apparently a reduced root growth.

1. Ewart, Phil. Trans. of Roy. Soc London, Vol. 198, Sect. B., 1905, p. 41; 1908, Vol. 199, p. 341; and annals of Botany, Jan. 1910, p. 85.
2. Pickering-Jour. Roy. Hort. Soc. London, Vol. 83, p. 487, and Woburn Fruit Farms Report No. 5, pp. 21-41.

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The water supply evidently affects the rapidly growing top more than it does root growth. It seems more likely that the rate of root growth is affected more by the amount of elaborated materials which reach them.

This Station has never been successful in getting peach or apple trees to grow in the greenhouse sufficiently well to permit conclusions to be drawn from them. A variety of pomegranate on account of its adaptability to greenhouse culture was chosen for experimentation. This variety has only a brief deciduous period. Some plants were started in pots in early December, 1907, shortly after they had finished the dormant period. A portion of the plants were cut back severely, the others left unpruned as checks. By observation it was found that during the period of rapid growth of the shoots, the root growth on the pruned trees was much smaller than on the unpruned trees. Figures 2 and 3 illustrate this fact. The pruned trees, however, held their leaves longer, and before their leaves had fallen the root growths of the pruned and unpruned trees were approximately equal. If pruning is a dwarfing process, then it seems highly probable that the reason will be found in the reduced nutrition of the roots due to the reduced leaf surface referred to in Table XVIII. It also seems probable that the dwarfing process would not be so great in sections where the growing seasons are long.

MOLAR CONCENTRATION OF LEAF AND FRUIT SAPS IN RELATION TO THE MOVEMENT OF WATER.

Pringsheim [1] seemed to find that in case of thick leaved plants such as *Sedum spectabile*, the young leaves were able to take moisture from the old dying leaves. Upon investigation he found that the young leaves had a higher sap concentration than the older leaves. While this seems true of plants with thick fleshy leaves, it is apparently not true of deciduous trees. Dixon and Atkins [2] found that the old leaves of lilacs and other trees had a larger sap concentration than the young ones. The work at this station verifies this finding in the case of peach and apple trees, as the following table will show:

1. Pringsheim-Jarb. f. Wiss. Bot. Vol. 43 (1906), pp. 89-144.
2. Dixon and Atkins—Sci. Proc. Roy. Dublin Soc. n. ser. 12 (1910), No. 25, pp. 275-311; and Sci. Proc. Roy. Dublin Soc. n. ser. 13 (1912), Nos. 16, pp. 219-222; 18, pp. 229-238; 19, pp. 239-246.

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TABLE XIX.—FREEZING POINT DEPRESSIONS OF SAP FROM OLD AND YOUNG LEAVES.

Date	Species	Freezing point depressions	
		Young leaves	Old leaves
April 31, 1908	Peach	1.980	1.937
April 31, 1908	Peach	1.954	1.927
June 5, 1908	Peach	1.210	1.725
June 5, 1908	Peach	1.185	1.417
June 6, 1908	Peach	1.310	1.511
June 11, 1908	Peach	1.967	1.989
June 11, 1908	Peach	1.558	1.931
June 16, 1908	Peach	2.083	2.195
June 17, 1908	Peach	1.615	2.290
June 12, 1913	Apple	1.202	1.849

The following table, however, shows that leaf sap has a much greater molar concentration than the sap from succulent fruits:

TABLE XX.—RELATIVE FREEZING POINT DEPRESSION OF SAPS FROM LEAVES AND FRUITS.

Variety	Fruit	Date	Depression leaf sap	Depression fruit sap
Downing	Gooseberry	June 2, 1911	1.380	1.005
Columbian	Gooseberry	June 2, 1911	1.410	.965
Wild Native	Gooseberry	June 2, 1911	1.530	1.050
Gano	Apple	July 19, 1911	1.880	1.465
Ben Davis	Apple	July 27, 1911	1.917	1.230
English Morello, (Green)	Cherry	June 5, 1911	2.708	1.425
English Morello, (Nearly ripe)	Cherry	June 5, 1911	2.708	2.243
English Morello, (Ripe)	Cherry	June 5, 1911	2.708	2.375
Montmorency (Green)	Cherry	June 10, 1913	2.370	1.260
Montmorency (Ripe)	Cherry	June 10, 1913	2.370	1.765
Perfection (Green)	Currant	May 26, 1911	1.130	.930
Perfection (Nearly Ripe)	Currant	June 15, 1911	1.130	1.110
	Strawberry	June 3, 1911	2.090	.745
	Black Raspberry	June 26, 1911	1.773	1.108
	Watermelon	Oct. 12, 1909	.882	.578
	Pumpkin	Sept. 28, 1909	.778	.648
	Cucumber	Sept. 28, 1909	.675	.525
	Gourd	Aug. 12, 1913	.927	.705
	Tomato	Aug. 12, 1913	.970	.875

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It would seem possible, then, that if the tree has insufficient water the fruit would tend to suffer, since the water would move by process of osmosis from the fruit to the leaves. There is a considerable amount of experience to indicate that this is true.

On a number of occasions twigs bearing leaves and fruit were cut from the tree, the basal end of the twigs paraffined and the fruit covered with paraffin without injuring the tissue (this can readily be done since paraffin has a very low specific heat). To prevent rapid wilting of the leaves, the twigs were then placed in a basement room where evaporation was slow. At the same time check twigs of the same variety and from the same tree, containing leaves but no fruit, were placed by the side of the twigs with fruit. The same was done with the annual plants. Following is a list of some of the experiments:

English Morello Cherries.

A. June 12, 1911, 3:00 P. M. Ripe fruit, paraffined, and left on stem with leaves. At 8:00 A. M. the following day the leaves were drying out but the fruit was not shriveled. At 6:00 P. M. the second day there was no shrinkage of fruit but the leaves were completely dried up. At 8:00 P. M. the fourth day the fruit was not shriveled. At 6:00 P. M. the second day there was no shrinkage of fruit but the leaves were completely dried up. At 8:00 P. M. the fourth day the fruit was not shriveled; leaves dry and curled.

B. Paraffined green fruit left on stem with leaves at 3:00 P. M. June 12, 1911. At 8:00 A. M. the following morning there was a slight shrinking of fruit; leaves still fresh. At 6:00 P. M. the second day the fruit was much shriveled and the leaves still fairly turgid. At 8:00 P. M. the fourth day the fruit was badly shriveled; the leaves were dying but were not dried out and curled.

C. (Check on A.) June 12, 1911, 3:00 P. M. Ripe fruit paraffined on stem with no leaves. At 8:00 A. M. the following day the fruit was not shriveled. At 6:00 P. M. the second day the fruit was not shriveled. At 8:00 P. M. the fourth day the fruit was not shriveled.

D. (Check on B.) June 12, 1911, 3:00 P. M. Green fruit paraffined on stem with no leaves. At 6:00 P. M. the second day the fruit was not shriveled. At 8:00 P. M. the fourth day the fruit was not shriveled.

Late White Cling Peaches.

A. June 12, 1911, 5:00 P. M. Young green fruit paraffined on twig with leaves. At 8:00 A. M. the day following, slight shriveling

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of fruit; leaves not wilted. At 6:00 P. M. second day the fruit shriveled; leaves still turgid. At 8:00 P. M. the fourth day the fruit was badly shriveled; leaves nearly dry. The fruit was very small and hence afforded only a small amount of water.

B. (Check on A.) No leaves; fruit paraffined at 8:00 A. M. Day following, no shriveling of fruit. At 6:00 P. M. the second day, fruit not shriveled. At 8:00 P. M. fourth day fruit not shriveled.

Sneed Peach.

A. June 12, 1911, 5:00 P. M. Fruit paraffined on twig with leaves. At 8:00 A. M. the following day there was no shriveling of fruit. At 6:00 P. M. second day fruit beginning to shrivel. The leaves are still turgid. At 8:00 P. M. fourth day the fruit was shriveled but leaves fresh. Fruits were large enough to furnish considerable water.

B. (Check on A.) No leaves, fruit paraffined. At 8:00 A. M. the following day there was no shriveling of fruit. At 6:00 P. M. the second day the fruit was not shriveled. At 8:00 P. M. the fourth day the fruit was not shriveled.

C. June 12, 1911, 5:00 P. M. Leaves on stem; no fruit. At 8:00 A. M. the following day, leaves wilted badly. At 6:00 P. M. second day the leaves were almost dry. At 8:00 P. M. the fourth day leaves were completely dry.

Grapes.

A. June 12, 1911, 5:00 P. M. Fruit paraffined and left on shoot with leaves. At 8:00 A. M. the following day the fruit was considerably shriveled. At 6:00 P. M. the second day the fruit was badly shriveled; leaves alive but wilted. At 8:00 P. M. the fourth day the fruit was completely shriveled and leaves dying.

B. (Check on A.) Fruit paraffined on shoot without leaves. At 8:00 A. M. the day following the fruit was not shriveled. At 6:00 P. M. the second day the fruit was not shriveled. At 8:00 P. M. the fourth day the fruit was not shriveled.

Downing Gooseberries.

A. June 13, 1911, 3:00 P. M. Fruit was paraffined and left on stems with leaves. At 8:00 P. M. the third day the fruit was turgid and the leaves almost dried out. At 3:00 P. M. the fifth day, the fruit showed no shriveling and the leaves were very dry.

B. (Check on A.) Fruit paraffined on twig without leaves. At 8:00 P. M. the third day the fruit was not shriveled. At 3:00 P. M. the fifth day the fruit was not shriveled.

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Perfection Currant.

A. June 13, 1911, 3:00 P. M. Fruit paraffined and left on stem with leaves. At 8:00 P. M. the third day the fruit was not shriveled and the leaves were almost dried out. At 3:00 P. M. the fifth day the fruit was not shriveled; but the leaves were completely dried out.

B. (Check on A.) Fruit paraffined, no leaves on twig. At 8:00 P. M. the third day fruit not shriveled. At 3:00 P. M. the fifth day fruit not shriveled.

Miscellaneous Species.

Thursday, May 18, 1911. Took twigs of Early Richmond, Montmorency, and English Morello cherries; of Juneberries; of apples; of peaches (Connett) and of strawberries and paraffined the fruits. On Saturday all fruits were shriveled, but the apples, peaches, cherries, and strawberries shriveled worse than the Juneberries. Early Richmond was worst shriveled of the cherries. Check fruit without leaves were not shriveled except strawberries and those were shriveled slightly perhaps on account of evaporation from stem bracts.

Saturday May 20, 1911. Cherries of the three above varieties were taken and prepared as above. On Monday they were shriveled but in the following order: Early Richmond, Montmorency, English Morello.

Tuesday, May 23, 1911, 12:00 M. Columbus and Downing gooseberries and currants were paraffined. By Wednesday afternoon, Columbus gooseberries were shriveling but no shriveling could be seen on Downing even by Friday noon. Fruit on twigs without leaves showed no shriveling.

Friday, May 26, 1911. Wild gooseberries were paraffined in the same way as other fruits with and without leaves. By Saturday fruits with leaves on the twigs showed some shriveling and by Monday considerable.

Thursday, May 25, 1911. Early Richmond cherries were paraffined at noon and by Friday no shriveling could be positively observed. By Saturday shriveling could be plainly seen. At the same time, gooseberries and currants showed like shriveling.

June 3, 1913, at 7:30 A. M. some Dyehouse cherry twigs with leaves and young green fruits were obtained. The fruits and cut ends of the twigs were coated with paraffin. The twigs were then placed in a moist basement room to wilt. By 10:00 A. M. the next day the young cherries had shriveled and become wrinkled. The leaves were turgid. At 10:00 A. M. the third day the leaves were

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beginning to roll; the green fruit had shriveled to the seed. At 5:00 P. M. the fourth day some leaves had turned yellow.

June 6, 1913, at 8:00 A. M. Montmorency cherry twigs with leaves and fruits (green and ripe) were gathered. The fruits and ends of the twigs were coated with paraffin. Some detached fruits (green and ripe) were also coated with paraffin; some were detached but received no further treatment. All were allowed to wilt. Twigs with leaves, but without fruit were put in as checks. At 5:00 P. M. the day following, the greenest paraffined fruits on the leafy twig showed most wrinkling; the red fruits were normal. The leaves were slightly more turgid than the leaves on twigs without fruit. The detached paraffined fruits were all turgid. Same was true of detached unparaffined fruits. 8:00 A. M. the third day all leaves considerably wilted. The green fruits attached to twigs were strongly wrinkled; the ripe fruits appeared normal. The paraffined detached fruits were all turgid. The green unparaffined detached fruits showed wilting. The ripe fruits are still normal. By 4:00 P. M. the fourth day, all the green cherries were very badly shriveled. The ripe fruits were still turgid. The half ripe fruits were shrinking some. All the leaves were much wilted. The detached paraffined fruits were normal. The detached, unparaffined green cherries had shriveled somewhat, but not nearly so much as those on the twig. The ripe fruits were normal.

On August 7, 1913, a piece of gourd vine about two feet long containing five large leaves and a fruit about five inches in diameter was placed by the side of a similar vine with no fruit. Two days later the vine with the fruit was fully turgid with the growing ends erect, while the one with no fruit was limp. On previous years, watermelons have shown the same phenomenon. Tomatoes under like conditions at the same time showed the same phenomenon but not so markedly.

It will be seen that in each case the leaves have been able to remove the water from the fruit and to remain alive when attached to the stem containing fruit much longer than when on stems without fruit. In fact in many cases peach leaves on stems containing fruit have never dried up as they normally do on detached stems, but have gradually turned yellow and fallen off much as in autumn leaf fall.

It is interesting to note that in the case of ripe cherries the water is removed from the fruit much more slowly than from green ones, as Figure 9 will show. The following table also shows the percentage of water removed in one day from green and ripe cherries:

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TABLE XXI.—LOSS OF WATER FROM CHERRY FRUITS ON A DETACHED TWIG.

Date	Condition of fruit	Moisture in fresh cherries	Moisture in wilted cherries	Percentage loss
June 14, 1913.....	..Ripe.....	86.54%	86.33%	0.002%
June 14, 1913.....	..Green.....	86.82%	84.02%	3.225%

Each of the figures given here is the average of three determinations where the range of error was narrow. Each of the wilted cherries, green and ripe, had been for one day in a moist basement room on detached twigs containing one leaf for each cherry. Of course the fresh cherries were different individuals from those whose moisture content was determined after wilting, but the highest moisture percentage for unwilted green fruit was 86.94 per cent and the lowest 86.27 per cent. In case of the green fruit the lower moisture content of those attached to the leafy twigs for a day must have been due to the removal of water by the leaves, since the fruits had been paraffined while on the twigs.

Reference to Table No. XX will show that ripe cherries have a much greater molar concentration than the green cherries, though still a smaller concentration than that of the leaves.

Perfection currants when nearly ripe and Downing gooseberries also lost their water more slowly than did most other fruits. Their fruits show a concentration more nearly equal to that of the leaves than do most other fruits. Columbus gooseberries and the wild gooseberries show a greater difference between the molar concentrations of the sap of fruit and leaves than do Downing and the leaves took the water from the fruit more conspicuously.

Results of some practical observations indicate that in the field at times a large leaf surface may reduce the size of the fruit, probably in this manner. Strawberries fertilized with nitrogen in the experiments carried on by the Missouri Experiment Station in South Missouri have made much larger leaf growth. During the dry season of 1911, and that of 1913, the fruit shriveled much worse on these large leaved (fertilized) plants than on plants of medium growth.

Mr. Frank Horsfall, formerly of the Missouri State Fruit Experiment Station, reports that during the sason of 1901, he advised a man who owned a peach orchard near Mountain Grove, Missouri, to cultivate it well in order to enable the trees to carry a good

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crop through the drought. The man did this and retained the foliage on his trees. There was a good set of fruit, but Mr. Horsfall reports that practically none of the fruit became larger than an egg, and many of them shriveled up on the trees. However, in an adjoining orchard in similar uncultivated soil, the leaves fell badly early in the summer but the fruit became large enough to be first class and a good crop was harvested. It seems probable that in this case the large foliage had simply kept the water exhausted from the fruit in much the same manner as the foliage on the twigs detached from the trees mentioned above.

Mr. D. A. Robnett of Columbia, Missouri, states that during the same season he cultivated well one of his orchards, while another was uncultivated. On the uncultivated orchard he harvested larger and better fruit. Other Missouri growers during the very dry season of 1901 had the same experience. Apparently on such a very dry season the large leaf surface was undesirable. This would probably be explained by the data given above.

In Bulletin 100 of the Pennsylvania Experiment Station, Stewart states that in the season of 1909 apple trees fertilized with nitrogen bore smaller fruit than trees not so fertilized. This was also a dry season. Stewart later apparently has come to the conclusion that the reason for this smaller fruit was the fact that the trees fertilized with nitrogen bore a heavier crop. It is not impossible, however, that both influences were at work. At any rate observations have been made on the Missouri Experiment Station grounds that indicate that the trees with the leaf surface reduced by severe pruning have borne larger fruit, even when they had a larger crop than the trees that had not been pruned so as to reduce the leaf surface. The following table shows the number of fruits, as well as the average size of the fruit, from pruned and unpruned Jonathan apple trees:

The trees were of the same age and had received the same treatment except as to pruning. The pruned trees were cut back rather severely on each of the two preceding years.

It will be seen that the pruned trees bore the larger fruit even though they had more fruits on the tree. A number of other pruned and unpruned trees have shown this same general result. Apparently the only explanation for this is that on these dry years the fruit suffered from having the large leaf surface with its ability to remove water from the fruits by means of the larger molar concentration of the leaf sap. It has not been possible, however, to increase the size of the fruit by removing part of the leaf surface.

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TABLE XXII.—COMPARATIVE NUMBER AND SIZE OF FRUITS ON PRUNED AND UNPRUNED JONATHAN APPLE TREES.

Date	Unpruned		Pruned	
	No. of Apples	Average Weight	No. of Apples	Average Weight
		grams		grams
Aug. 5, 1913.....	192	38.67	857	70.50
Aug. 5, 1913.....			1149	62.00
Aug. 9, 1913.....	436	60.30	1216	61.00
Aug. 15, 1913.....			656	73.65
Aug. 15, 1913.....	1424	68.16	822	81.33
Aug. 15, 1913.....	468	62.56	449	63.72
Aug. 15, 1913.....	51	72.87	471	82.21
Average.....	514	63.7	803	72.3

During the very dry season of 1913 in picking peaches on fertilized plots at Brandsville, Missouri, Mr. A. J. Heinicke observed that previous to August 6 the fruit on the trees that had been fertilized with nitrogen and showed a very greatly increased foliage was wilted. This could be observed both by the appearance and the feeling of the peaches. On August 6, there was a shower large enough to increase the humidity of the air and to greatly reduce the evaporation, but probably not large enough to affect the available supply of moisture in the soil. On the next day, the fruits on the fertilized trees were as turgid as those on the unfertilized trees. In all cases the fruits on the trees fertilized with nitrogen were smaller, but in nearly all cases the crop was larger so the difference in size could not positively be attributed to the removal of water from the fruit by the leaves. It seems probably, however, that since the trees had been fertilized for three or four years with nitrogen and were larger and much stronger than the unfertilized trees they should have been able to carry a larger crop of fruit. In one case, twelve trees that had been receiving nitrogen for three seasons, bore an average of 314 peaches or 40 pounds to the tree. The average size of the peaches was .128 pounds. Eight unfertilized trees bearing an average of 305 fruits or 45.4 pounds to the tree had fruit, the average size of which was .149 pounds. In this case the difference in size could not have resulted from the difference in the size of the crop.

During the season of 1911, which was rather dry in South Missouri a partial crop of fruit was borne in the orchard of the Ozark

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Fruit Farm Company at Brandsville, Missouri, on some trees that had been pruned severely in the spring of 1910 and on some trees that had been fertilized heavily with ammonium sulphate at the same time. The fertilized trees made a very rapid growth with large leaves during the summer of 1911, and the pruned trees also continued to grow vigorously during that season. The pruned trees bore more fruit, three and one-fourth bushels on nine trees against four bushels on thirty fertilized trees. It required 122 fruits from the pruned trees to make a bushel against 209 from the fertilized trees. The pruned trees evidently had a much smaller leaf surface in proportion to their root system and probably also a much reduced resistance to the sap flow because much old wood had been cut off, thus forcing the fruiting twigs much nearer the root system.

At East Bloomfield, N. Y., in 1913 an orchard belonging to Mr. George A. Wheeler had been well cultivated and fertilized heavily. It carried a vigorous appearing foliage late into the season, which certainly indicated a thrifty condition of the tree, yet the fruit on these trees was exceptionally small. In a number of orchards observed near the Lake Ontario Region, where it would be expected that the humidity would be much greater and the evaporation less, the fruit was unusually large, though the trees were bearing as large or larger crops than those borne by the Wheeler orchard.

These instances are not offered as positive proof that a heavy foliage may reduce the size of the fruits on dry years on account of the greater osmotic strength of the leaves, but they certainly seem to indicate that such is the case.

Even in a season of drought with rain at the ripening time, it seems possible that trees with a reduced foliage are more likely to develop fruit of a normal size than those with a large leaf surface. It is not claimed, however that this is positively proven.

In discussing the removal of water from the fruits by the leaves, it has been assumed that this movement took place because of the greater osmotic concentration of the sap of the leaves. In order to explain the movement of water in this slow way in the direction of the greater molar concentration, it is not necessary to assume that the columns of water have as large tensile strength as they would in the case of the upward movement of the sap, if the molar concentration of the sap of the leaves is considered the force that brings about the movement. In this case the movement is so slow that it could probably take place by osmosis from cell to cell, or probably, as a result of the difference in vapor tension. Assuming no other influences, the vapor

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tension in the leaves must be less than that of the solution with the lower molar concentration in the fruit. Of course it is possible that the force of imbibition affects the vapor tension in such a way that there would be little or no difference resulting from the different molar concentrations. To the best of the writer's knowledge, the extent of this influence has not been successfully determined.

In order to give more positive evidence as to whether or not the movement of the water from the fruit to the leaves is due to the greater osmotic concentration, tomato plants were grafted by the approach method. After a union was perfected they were uprooted and the root system of one of the plants placed in water, and that of the other in a cane sugar solution with a concentration much greater than that of the sap of the plants. The roots were kept in this way for two days. Then the plant whose root system had been kept in water was placed in a moist chamber with a paraffin paper cover that was slit so that the leaves of the other projected above the paraffin paper cover. The plants were kept in this way until the one whose roots had been kept in sugar solution began to show some wilting. They were then removed and it was found that the one in the moist chamber whose roots had been kept in water solution was wilted worse than the exposed leaves of the plant whose roots had been kept in the sugar solution, as Plate 12 will show.

It seems conclusive then that the water had moved from the plant in the moist chamber through the graft union to the exposed leaves of the other plant. It indicates that the water moved in the direction of the greater osmotic concentration. While we have no check to tell the difference in concentration, (the osmotic concentration was not determined with these particular plants) we have by a similar method more than doubled the concentration of the leaf sap of tomatoes.

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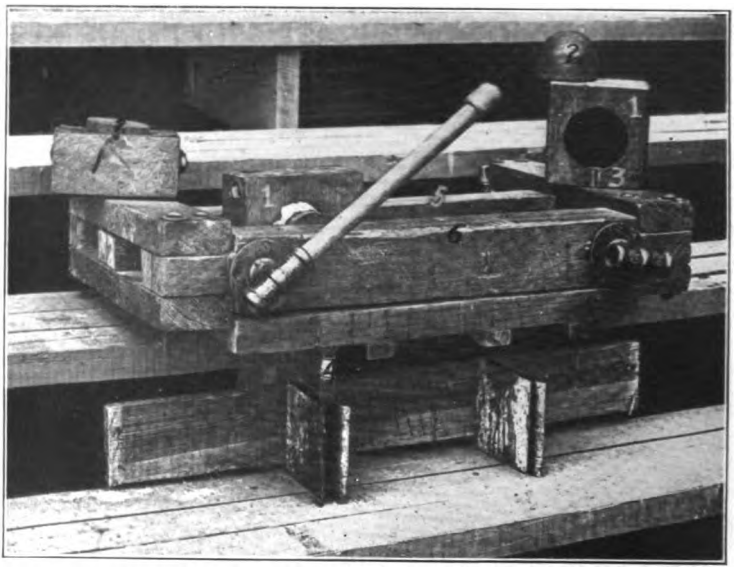

PLATE 1.

Apparatus for expressing plant juice. The two blocks between which the plant tissues were pressed are shown at 1 and 2. The juice escaped through a hole, 3, in block 1. The plant tissue was placed between the blocks 1 and 2, which in turn were placed between the pieces of movable 4 inch x 4 inch pieces which are drawn together by bench screws.

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PLATE 2.

Comparative root growth of an unpruned pomegranate and one severely pruned two months previously. Note the larger number of roots projecting through the soil of 2, the unpruned plant.

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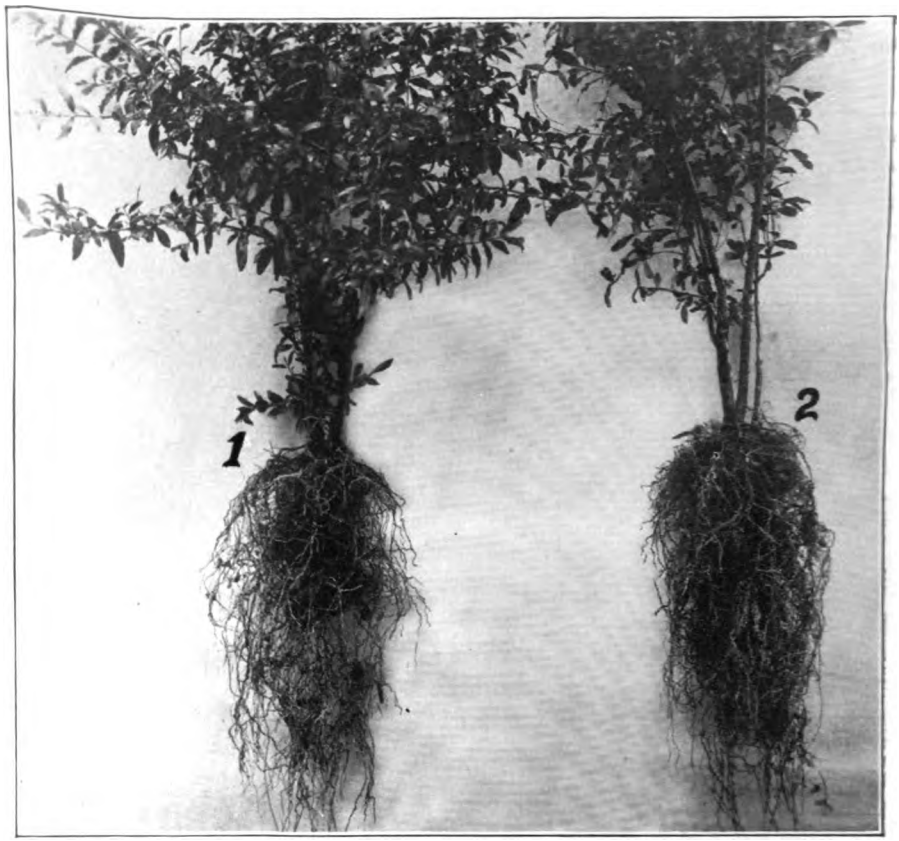

PLATE 3.

Comparative root growth of an unpruned pomegranate and one severely pruned four months previously. The root system of 2, the unpruned plant, is much larger than that of the pruned plant.

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